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AMRL-TDR-63-75

ENVIRONMENTAL POLLUTION BY MISSILE PROPELLANTS

TECHNICAL DOCUMENTARY REPORT No. AMRL-TDR-63-75

AUGUST 1963

BIOMEDICAL LABORATORY
6570th AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

Contract Monitor: James R. Prine, Major, USAF, VC
Project No. 6302, Task No. 630204

(Prepared under Contract No. AF 33(616)-7801 by
Walter W. Heck, Morris E. Bloodworth,
William J. Clark, Dale R. Darling, and William Hoover
Texas A. and M. Research Foundation, College Station, Texas)

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FOREWORD

This study was initiated by the Biomedical Laboratory of the 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division. The research was administered by the Texas A. and M. Research Foundation, College Station, Texas, under Contract No. AF 33(616)-7801. Actual research was performed by the Biology, Plant Sciences, and Soil and Crop Sciences Departments of the A. and M. College of Texas, College Station, Texas. Dr. Walter W. Heck, of the Plant Sciences Department, was the principal investigator. James R. Prine, Major, USAF, VC, of the Toxic Hazards Branch, Physiology Division, was the contract monitor for the 6570th Aerospace Medical Research Laboratories. The work was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630204, "Environmental Pollution." The research was started in January 1962 and was completed in November 1962. The authors acknowledge the assistance of Mr. Lloyd Hayes in many facets of the technical work involved in this report. This report is catalogued by the Texas A. and M. Research Foundation as Project RF-280.

The authors were in charge of the following areas of investigation: Dr. Walter W. Heck, Plant Physiologist, the effects of propellants on plant growth and development; Dr. Morris E. Bloodworth, Soil Physicist, the effects of propellants on soil and soil structure; Dr. William J. Clark, Limnologist and Phycologist, the effects of propellants on aquatic organisms; Mr. Dale R. Darling, Research Assistant, experimental work on plant growth and development; and, Mr. William Hoover, Research Assistant, experimental work with soils.

ABSTRACT

Experimental procedures were developed to study the effects of hydrazine, unsymmetrical dimethylhydrazine (UDMH), pyridine borane, and nitronium perchlorate on plant growth and development, soil and soil structure, and aquatic organisms. Plant growth and development research included: seed germination, seed growth, and treatment of plants in water culture, by sprays, and with the test chemicals as air pollutants. Under the conditions used in this study, the four chemicals do not appear to be important environmental contaminants in relation to plant growth and development. Both UDMH and hydrazine are strongly adsorbed or decomposed on clay particles. Montmorillonite and kaolinite clays, as well as the test soils, seem to accelerate the decomposition of the UDMH and hydrazine. Pyridine borane was adsorbed on the test soils but apparently was not adsorbed on the pure clays. The aquatic life was very sensitive to the three organic compounds and to some extent to the perchlorate ion.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

Jos M Quashnock

JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biomedical Laboratory

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I

INTRODUCTION

This report is the second on the environmental effects of missile propellants. The first report (ref. 12) covered preliminary experiments with 21 test chemicals. These compounds were initially submitted to a rather comprehensive literature survey which delineated those compounds which had been well studied from those which had been poorly investigated or not studied at all. The research program covered four major areas: an investigation of the effects of the test chemicals on the soil microflora; the interaction of each test chemical with a specific soil type; the effects of the test chemicals on plant growth and development; and, the effects of the test chemicals on aquatic life.

The present research was developed to further explore the potential environmental hazards of four specific fuel components. These four chemicals were investigated to delineate more accurately their toxicity to several environmental components: soil and soil structure, plant growth and development, and aquatic life.

II

RESEARCH

A. General Areas of Study

This project has been subdivided into three major areas of investigations.

1. Soil Studies

Soil research was directed toward studying the effect of the mineralogical composition of two clays and six soils on the adsorption of the test chemicals. In addition some soil column work with specific soil types was run to determine their adsorptive capacity for the four test chemicals.

2. Plant Studies

These were divided into two major areas of investigation.

a. The effects of specific chemical concentrations on the germination and subsequent growth of three species of plants.

b. The effects of specific chemical concentrations on the growth and development of young plants. These research areas included the application of the chemicals as water culture contaminants, as sprays and as air fumigants.

3. Aquatic Studies

Three studies were conducted to determine the toxicity of the four test chemicals on five species of fish: a TLm (median tolerance limit) series; the effect of age of test solution on toxicity; and, timed exposure tests. Limited studies were also conducted on the toxicity of the test chemicals to Daphnia, (an aquatic microcrustacean) and to Chlorella (a green algae).

B. General Experimental Procedures

1. Organisms To Be Studied

a. Plants

1) Cucurbita pepo, (L.) Alef. (condensa, Bailey) var. melopepo - summer brush squash

2) Glycine max, (L.) Merr. - Soybean

3) Gossypium hirsutum, L. var. Austin - Cotton

4) Vigna sinensis, (Torner) Savi: - Cowpea, extra early blackeye

5) Zea mays, L. - Corn, single cross 325 x 203, lot 215 - 4 + 5L composite sample, May 1962, Texas A & M Seed Foundation, College Station, Texas

6) Arachis hypogaea, L. - Peanut, Spantex

7) Cichorium endivia, L. - Endive

8) Medicago sativa, L. - Alfalfa

9) Phaseolus vulgaris, L. - Pinto bean

b. Aquatic Organisms

Organisms studied include an algae Chlorella pyrenoidosa, (Chick) a microcrustacean Daphnia pulex, (de Geer) and the following fish: goldfish, Carassius auratus, (Linnaeus); green sunfish, Lepomis cyanellus, (Rafinesque); bluegill, Lepomis macrochirus, (Rafinesque); channel catfish, Ictalurus punctatus, (Rafinesque); and, large mouth bass, Micropterus salmoides, (Lacepede).

The bluegill, channel catfish, and large mouth bass were obtained from the Huntsville Hatchery of the Texas Game and Fish Commission; the goldfish and green sunfish from a local bait breeding farm.

2. Soil Types

a. Soil and Soil Structure

Six soils, two relatively pure clay types and a sand were used in these studies. Table 1 lists the texture and more important physical-chemical properties of the soils and "pure" clay materials which were used. Appendix C provides X-ray diffraction patterns for the soil clays.

TABLE 1

Texture and Important Physical-Chemical Properties of the Soils and Clay Materials

Soils	Texture			Name	% Water*	pH	Elect. Cond. (mmhos/cm)	% Org. Matter	ppm N as NO_3	ppm Sol. P	Exchange Capacity (meq/100 gm)	Clay Fraction/
	%Sand >20μ	%Silt 20μ-2μ	%Clay <2μ									
Houston	4	41	55	Clay	10.44	7.8	0.48	4.74	<1.0	3.6	65	high
Lufkin	32	18	50	Clay	6.21	5.7	0.36	1.15	<1.0	1.0	29	med. to high
Aiken	36	38	26	Loam	6.87	6.8	0.33	4.21	18.3	3.0		undeter.
Montezuma	22	22	56	Clay	5.50	5.5	0.38	2.75	8.4	8.2		high
Hanford	89	8	3	Loamy Sand	0.44	7.1	0.24	0.38	2.4	26.1		none
Yolo	34	38	28	Loam	2.65	6.8	0.35	2.64	6.4	27.3		low
Special Soil Component Materials												
Washed Sand	100	0	0	Sand	-	-	<0.01	<0.01	<0.01	<0.01		
Montmorillonite	0	0	100	Clay	16.30	7.7	1.02	0.23	8.0	2.0	92	high
Koalinite	0	0	100	Clay	1.17	4.2	0.34	0.00	1.5	45.5	3.4	

* Air dry samples.

/ The relative amount of montmorillonite in the clay fraction. Determined by X-ray diffraction. See Appendix C.

The sample of Houston black clay was collected at a site 12 miles east of Temple, Texas. The sample was taken at a depth between 16 and 30 inches from a virgin meadow with native blue-stem vegetation. The sample of Lufkin loam was collected 1/4 mile west of the Texas A & M College dairy. The sample was taken at a depth between 10 and 20 inches in the B2 horizon. The texture of the B horizon of the Lufkin loam is a clay (montmorillonite). The sample area has a sparse stand of post-oak trees and native shortgrass vegetation. A more detailed chemical, physical, and mineralogical analysis of both soil series are available from the Soil and Crop Sciences Department of the A & M College of Texas.

The Aiken, Hanford, Montezuma, and Yolo samples were taken by the Air Force from a missile test area. The samples were forwarded to Texas A & M College from the McClellan A.F.B., Sacramento, California. Further information on the Aiken soil series can be found in the U.S.D.A. Placerville Area, California

Soil Survey, Series 1927, No. 34. Information on the Hanford soil series can be found in the U.S.D.A. Madera Area, California Soil Survey, Series 1951, No. 11. Information concerning the Yolo soil series can be found in the U.S.D.A. Contra Costa County, California Soil Survey, Series 1933, No. 26. The Montezuma soil series is described in the U.S.D.A. Santa Cruz, California Soil Survey, Series 1935, No. 25.

The "pure" montmorillonite clay was purchased as Panther Creek Bentonite from the American Colloid Co., Skokie, Illinois.

Bulk kaolinite clay was purchased from the Georgia Kaolin Co., Elizabeth, New Jersey.

The washed sand was originally beach sand that had been washed repeatedly until all of the organic matter, clay, and silt were removed.

b. Plant Studies

Except for the seed germination study, all plants were germinated and grown to the test age in the greenhouse in a peat-perlite potting mix.

The peat-perlite potting mix is an unpublished modification of the standard mix used last year (ref. 12) both of which were developed by the Department of Floriculture at Texas A & M College (ref. 7). The mix is made from dry baled Sphagnum horticultural peat, horticultural grade perlite and various fertilizers (Appendix A).

3. Water and Nutrient Solutions

a. A 1.0 N sodium acetate-acetic acid buffer at pH 5.4 was used to extract the test chemicals from the various soil fractions. Ethyl alcohol (95%) was used to wash the excess chemicals from the soil samples prior to extraction. Distilled water was used when preparing water solutions of the test compounds.

b. Seed germination and growth studies utilized distilled water with no added nutrients.

c. Plants were grown in the greenhouse until moved to the growth chambers for testing purposes. In the greenhouse, the plants were watered every other day with a full strength Hoagland's solution (Appendix A) containing 15.0 grams of Mortons Soil Drench C (methylmercury dicyandiamide-2.2%, a fungicide) per 72 liters of

solution. The water culture experiment used full strength Hoagland's solution in distilled water. This solution was changed after five days. All other experiments were of short duration requiring no further watering other than in the greenhouse.

d. Aquatic Studies

A standard reference water was used for all fish experiments (Appendix A). The standard water was prepared in 50 liter lots in plastic containers. Several modifications were made in the procedure as outlined due to a variation in the final pH from that given in the procedure. Where the final pH was too high, carbon dioxide was bubbled into the water to lower the pH and then aeration was continued to an equilibrium pH. In most experiments aeration was stopped at a pH of 7.0 to 7.2 and stock solutions 5 and 6 were added. Aeration was then continued until an equilibrium pH was attained. The pH of the standard reference water, as prepared under the above procedures, stabilized at 8.2 rather than at 7.9.

Daphnia experiments were run with tap water which had been aerated to remove the chlorine. The standard reference water was not used due to mortality of the control organisms in this water source.

Algal cultures were grown in a modified Knops medium (Appendix A).

4. Chemicals and Their Use

a. Physical and Toxicological Properties

1) Hydrazine (NH_2NH_2): purity (95+ percent as obtained from the manufacturer); vapor pressure - 14.38 mm at 25°C and 19.3 mm at 30°C (ref. 1); density at 25°C - 1.011. Hydrazine is a volatile liquid which is highly toxic. It has a MAC of 1 ppm in air (ref. 18).

2) UDMH (1,1-dimethylhydrazine - $(CH_3)_2NNH_2$): purity (close to 100 percent - was redistilled before use and the fraction boiling at 63.5°C was used in many of these experiments); vapor pressure - 157.0 mm at 25°C (ref. 18); density at 25°C - 0.786. UDMH is a volatile liquid which is highly toxic. It has a MAC of 0.5 ppm in air (ref. 18).

3) Pyridine borane ($C_5H_5N:BH_3$): purity (about 100 percent as obtained from the manufacturer - see note); vapor pressure -

0.1 mm at 25°C (ref. 4); density at 25°C - 0.92. The toxicological properties are undetermined, but are less than borane hydrides such as diborane with an MAC of 0.1 ppm in air (ref. 11).

4) Nitronium perchlorate (NO_2ClO_4): purity (95 percent minimum from the manufacturer); vapor pressure - 0.05 mm at 25°C. This chemical is very hygroscopic; therefore, all transfers must be made in a dry box, and samples should be stored in a desiccator. Hydration of samples results in the formation of nitric and perchloric acids. When these acids are found within the nitronium perchlorate as trace contaminants, they cause the nitronium perchlorate to exhibit some shock sensitivity. Other dangers arise from the toxicity of lower nitrogen and chlorine oxides.

5) The number of replaceable hydrogen ions in UDMH, hydrazine and pyridine borane is uncertain; thus, all soil calculations are based on a single replaceable hydrogen ion in each of the test compounds. The millequivalent values then are the same as millimoles.

b. Mixing and Application of Test Chemicals

The chemicals were prepared as water solutions in all except the plant fumigation experiments. In the fumigation experiments the three liquids were used as the pure chemical while a 70 percent perchloric acid solution was used in place of the nitronium perchlorate. For the water solutions the three liquids were added on a volume/volume basis of chemical to water (v/v basis) and not on a weight basis. No correction was made for the purity of the chemical. The nitronium perchlorate was added on a weight/volume (wt/v) basis to water. The chemicals were added to distilled water at high concentrations and then the concentrates were added directly to distilled water, the plant nutrient solution or to the fish solution depending upon the experiment being performed.

c. Concentrations of Chemicals Used

1) Soil and Soil Structure

All chemicals were tested by application to soils in distilled water solutions. UDMH was tested at 10, 100, 500, 1000, 5000, and 10,000 ppm (v/v); hydrazine at 500 and 1000 ppm (v/v); pyridine borane at 500 and 1000 ppm (v/v); and, nitronium perchlorate at 500 ppm (wt/v).

2) Plant Growth and Development

Seed germination and development: The chemicals were used at concentrations of 0, 1, 10, 100 and 1000 ppm (v/v except for nitronium perchlorate) in distilled water. Water culture study: The chemicals were used in this study at 0, 1, 10, 100, 300, 600 and 1000 ppm (v/v except for nitronium perchlorate) in complete Hoagland's solution. Spray studies: The chemicals were applied in a distilled water solution at 0, 2000, 6000, and 10,000 ppm (v/v except for nitronium perchlorate) with a 0.1 percent concentration of a spreader activator present to reduce leaf surface tension. Fumigation studies: The chemicals were applied as vapor in air mixtures at various concentrations.

3) Aquatic Life

Concentrations of all test chemicals were used at from 1 to 800 ppm (v/v except for nitronium perchlorate) in the standard reference water for fish. Specific concentrations for any given experiment are given in the aquatic research section. All solutions were made immediately before the addition of the test organisms. Chemicals were added to chlorine-free tap water in the Daphnia studies and to the Knops medium for the algal research.

5. Analytical Chemical Determinations

a. General

Quantitative colorimetric procedures were used for the detection of the various test chemicals. Procedures and absorption curves were developed using a Beckman DB Spectrophotometer but the standard curves used in routine analysis were developed using a transistorized model of a Bausch and Lomb Spectronic 20.

A standard curve was developed for each test chemical in each of the four testing solutions: distilled water, standard reference water (fish), complete Hoagland's solution (plants), and in sodium acetate buffer (soils).

b. Hydrazine

The procedure used is a modification of the one by Watt and Chrisp (ref. 21), see Appendix B. The standard curves were developed using the 460 mμ absorption band. This gave a straight line function over the concentration range of 0-4 ppm (v/v basis).

The standard curves were obtained by taking the average of three standard curves which were run at different times. The

curves used in developing the average standard curve did not deviate from the average value more than ±2%.

c. UDMH

The procedure for the determination of UDMH was developed by Pinkerton et al. (ref. 17). Standard curves were developed using the 500 mμ absorption band.

Difficulties were initially encountered in developing a standard curve. This was traced to a bad lot of the test reagent trisodium pentacyanoamino ferrate (TPF). When the reagent was replaced, the standard curve was readily replicated over the concentration range of 0-100 ppm UDMH (v/v basis).

d. Pyridine borane

Pyridine borane is quite resistant to hydrolysis in water; therefore, it is possible to analyze for the compound intact, rather than for pyridine or boron individually. Phosphomolybdic acid (PMA) has been used for the quantitative determination of several boron hydrides. A review of available literature indicates that prior to this report a standard method had not been developed for pyridine borane. However, Hill did suggest that the method might be modified for use with pyridine borane (ref. 13).

The reaction of PMA with pyridine borane produces a yellow color which is pH sensitive. At a pH of 1.7-2.0, the reaction of PMA with pyridine borane produced a stable pale yellow color with maximum color development in 14 minutes which remained stable for more than 10 minutes. The adsorption curves for both the PMA and the PMA-pyridine borane complex showed a maximum at 515 mμ.

When the yellow color of the complex was allowed to develop to its maximum (15 minutes) and the pH then raised to 5.4, the yellow color turned blue with the maximum color developing within 5 minutes. This color remains stable for more than 10 minutes. At a pH of 5.4 the blank turned from yellow to clear. An absorption maximum for the blue color was found between 710-732 mμ, but 625 mμ was selected for running the test samples since it was a plateau region of the curve.

The procedure used for determining pyridine borane gives reproducible linearity between 0 and 100 ppm of pyridine borane (v/v basis) for all test solutions. Reagents: (1) Color-developing solution (PMA); dissolve 12.5 gms of phosphomolybdic acid in

water, add 2 ml of concentrated hydrochloric acid and make to 500 ml, filter and use the filtrate. (2) Sodium acetate buffer: make a 1N sodium acetate solution and add acetic acid to bring the pH to 5.4. Procedure: place 1 ml of the test solution of pyridine borane into a small flask; add 2 ml of the PMA color-developing solution, mix and let the color develop for 15 min.; add 3 ml of the buffer solution, mix and let the color develop for 5 min.; the OD is read at 625 mμ. Note: When using the sodium acetate buffer in the soil studies, the pH must be lowered to 2.0 for maximum color development.

Many reducing substances apparently react with the color-developing reagent. Thus, when this procedure was used to check pyridine borane concentrations in solutions where fish had been kept, apparent concentrations as much as double the initial concentrations were found. This procedure as outlined is applicable only to the four standard solutions and would have to be re-evaluated for use with plant or animal products or extracts.

The initial supply of pyridine borane, obtained from Callery Chemical Company, was an opaque liquid with a white granular precipitate. The original standard curves and all experiments, with the exception of the plant fumigation studies, were done utilizing this batch of reagent. A second bottle purchased for the fumigation study was a clear, light yellow solution with no precipitate. Due to the difference in the physical characteristics a new standard curve was run for the second chemical batch. This curve was 25 percent higher than the initial curve. Thus the purity of the first supply is questionable as is the absolute purity of the second batch.

e. Nitronium Perchlorate

The procedure used was a modified methylene blue analysis for the perchlorate ion as described by Baltz (ref. 2). The reason for the analysis of the perchlorate ion is that nitronium perchlorate breaks down in aqueous solution according to the following equation to form an equimolar mixture of nitric and perchloric acids (ref. 5):

$$NO_2ClO_4 + H_2O \longrightarrow HNO_3 + HClO_4$$

Thus the nitrate and perchlorate ions can be analyzed separately. Since nitrate is a normal component of soils and the plant and fish test solutions, it is difficult to determine small nitrate variations. In addition nitrate is a normal nutrient for plants and a normal constituent in aquatic areas. Thus no toxicity would be expected due to the nitrate ion, there might even be a slight stimulation of growth.

The test used is based on the fact that perchlorate ion forms an insoluble complex with methylene blue in water. This complex is soluble in chloroform. Development of the complex appears to be almost immediate upon contact of the methylene blue with perchlorate ion. Also, the complex formed appears to go into solution in chloroform almost immediately so that timing is not important. The final color developed is stable for over 15 minutes.

The procedure used for determining the perchlorate ion is a simple one which gives reproducible linearity between 0-100 ppm of perchlorate (wt/v basis) for all test solutions. The absorption spectrum of the methylene blue solution gave a maximum at 740 mμ but 630 mμ was used in these determinations since it was a plateau region of the curve. Reagents: 1). Perchlorate ion - prepare a standard solution of potassium perchlorate by dissolving 1.3935 gms of $KClO_4$ in a liter flask and making to volume. This solution contains 1000 ppm of ClO_4 ion. 2). Methylene blue - dissolve 0.5 gm of methylene blue in water and make to 1 liter. 3). Chloroform - USP grade.

Procedure: Place one ml of the perchlorate standard plus one ml of the methylene blue solution in a small beaker and swirl to insure mixing. Add 25 ml of chloroform and mix well. Transfer a portion of the chloroform layer containing the colored complex to a cuvette and read at 630 mμ against a blank prepared by using one ml of distilled water in place of the perchlorate standard.

6. Chemical Breakdown

Audrieth and Ogg (ref. 1) state that aqueous solutions of hydrazine readily undergo auto-oxidation to yield hydrogen peroxide. Water solutions of hydrazine are susceptible to direct oxidation by the atmosphere; whereas, acid solutions of hydrazine are stable and less easily decomposed. Therefore, dilute solutions of hydrazine deteriorate readily on contact with the atmosphere. The products obtained are dependent on the initial pH of the solution, dilute solutions in water decomposing as follows:

$$3N_2H_4 \longrightarrow 2NH_3+2N_2+3H_2$$

As the pH increases, the amount of N_2 and H_2 produced increases and NH_3 decreases; thus, at a high pH the catalytic decomposition of hydrazine, in dilute aqueous solutions to N_2 and H_2 becomes the limiting reaction.

The decomposition of hydrazine has been studied and is an important consideration when developing reproducible standard curves. It is also important to know the rate of breakdown in the several test solutions in order to adequately discuss the potential environmental effects of the test chemicals. The rate of breakdown would also be important when trying to maintain a constant concentration in the various test solutions.

Although no information was found regarding the stability of UDMH in aqueous solutions, our experience would suggest that it is reactive in aqueous media and readily breaks down. The vapor pressure of UDMH is high and loss would be anticipated from the aerated fish solutions in addition to chemical breakdown.

Neither hydrazine nor UDMH show appreciable decomposition when prepared in distilled water which is free of trace metal impurities. Thus the extent of decomposition under natural conditions needs to be determined.

Pyridine borane and nitronium perchlorate are apparently stable in aqueous solutions. Pyridine borane was included in these studies as a check chemical but the nitronium perchlorate solution was not tested for loss of the perchlorate.

a. Procedure

Duplicate solutions of 10 and 100 ppm (v/v) of hydrazine, UDMH and pyridine borane were prepared in distilled water, standard reference water (fish solution) and in complete Hoagland's solution. (UDMH was not run in the standard reference water.) Five hundred ml of each test solution was placed in a 600 ml beaker and left uncovered during the experimental run. A second series was developed using only distilled water and the standard reference water. This series had the test solutions aerated at a fairly rapid rate during the course of the experiment.

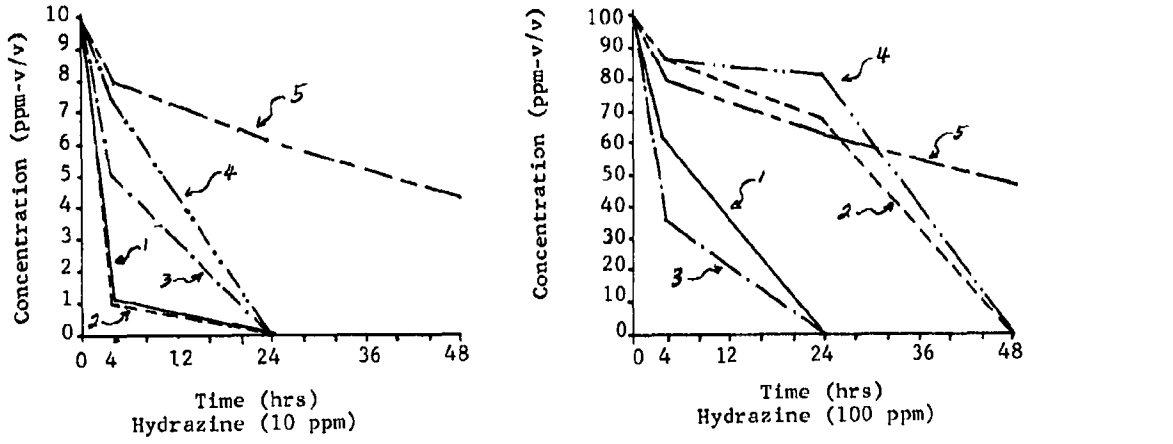
The starting times of the various experiments were arranged so samples were taken and read colorimetrically from the standard curves as quickly as possible. Other samples were taken and analyzed immediately at 4, 24, and 48 hours, at which time the experiments were terminated.

At zero time, a second set of samples was taken and stored in capped glass or plastic bottles. These samples were analyzed 24 hours later to determine if the samples could be stored for a period of time without deterioration.

b. Results

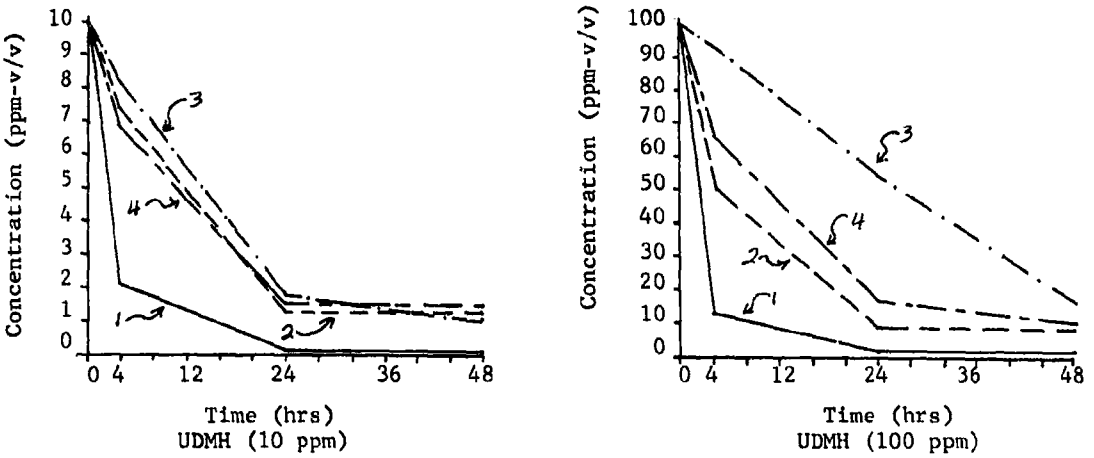
The results of these experiments for hydrazine and UDMH are presented graphically (Figures 1 and 2) for both the 10 and 100 ppm solutions. Pyridine borane did not decompose under any of the experimental conditions and therefore the curves for this chemical are not shown. Hydrazine and UDMH both show extensive decomposition under the various experimental conditions within the 48-hour test period. A comparison of the aerated and unaerated UDMH test runs suggests that the greater loss under aerated conditions is due mainly to oxidation and not to vaporization of the test compound; however this was not tested.

Analysis of the samples which were stored for 24 hours showed from 25 to 50 percent less decomposition in these samples than in those kept in open containers or where the solutions were aerated.



LEGEND:
1. ———————— distilled water, aerated
2. ---------- distilled water, unaerated
3. —·—·—·—·— standard reference water (fish solution), aerated
4. —··—··—··— standard reference water (fish solution), unaerated
5. —— – —— – – Hoagland's solution, unaerated

Figure 1. Breakdown Rates of Two Concentrations of Hydrazine in Various Test Solutions.



LEGEND: 1. ———— distilled water, aerated
2. -------- distilled water, unaerated
3. —·—·—·— standard reference water (fish solution), aerated
4. —— — — Hoagland's solution, unaerated

FIGURE 2. Breakdown Rates of two Concentrations of UDMH in Various Test Solutions.

7. Growth Chambers

Four plant growth chambers have been utilized in our young plant studies. Details of chamber construction were presented in the earlier report (ref. 12). These chambers were designed for fumigation or air pollution studies and thus use a dynamic air flow system with no air recirculation. The chambers are 48 inches wide x 30 inches deep x 42 inches high with a total volume of 35 cubic feet. They are constructed of a wood frame covered with an 1/8 inch clear plexiglas on the top and sides. The chambers are equipped with two 2-inch air inlets and two 2-inch air outlets. The outlets have machined orifice plates which fit snugly over the outlet tube for measurement of air flow.

Night temperatures are maintained at 71.5 $\pm$ 3.5°F with room air conditions. Day temperatures are maintained at 84 $\pm$ 4°F with a thermostatically controlled heater in the main inlet duct. Although no attempt was made to control humidities, the chambers maintained a 65 $\pm$ 13% night humidity and a 57 $\pm$ 13% day humidity as recorded with a wet-bulb thermometer. The plants received a twelve-hour day by use of artificial lighting. The light bank

was composed of twenty-four 8-foot powergroove fluorescent lamps and 6-150 watt incandescent lamps. The bank of lights was suspended over the four chambers and gave a maintained light intensity of 1000 to 2000 fc on the floor of each chamber.

C. Soil and Soil Structure

1. Introduction

Cation adsorption phenomenon on clays has been extensively explored. However, little has been done with the adsorption of organics and large molecules on clays. Gieseking (ref. 9) published some of the first work on the adsorption of organics on clays in 1939, but this phase of soil chemistry and physics has been slow to develop. Some of the more apparent reasons for the lack of such work are: 1) lack of procedures for studying organic and large molecule adsorption on clays; 2) lack of equipment and techniques to characterize the more complex problems of adsorption; and, 3) lack of immediate economic importance.

The original problem concerned with soils was to find general information on structure, runoff, and the amount of leaching for twenty-one chemicals found in missile propellants or missile exhausts. The information gained in the first study stressed the need for basic studies in order to better interpret the results that were obtained (ref. 12).

It was thought that more fundamental and meaningful information could be found if the interrelationship between the four chemicals and the clay fraction of soils was studied. The clay fraction of a soil has two qualities that are of utmost importance in a study of this kind. First, the shape and the small size of clay particles which results in a very high surface area for a given weight of clay. Colloidal clay has approximately 1,000 times the specific surface area of very fine sand. (The specific surface area is given as the square centimeters of surface area per unit volume (in cc) of material). The second unusual quality of clays is their ability to adsorb large amounts of positively charged ions by the process of cation exchange. As an example, montmorillonite-type clay has a high exchange capacity of approximately 100 meq/100 gm of soil, while kaolinite has a relatively low exchange capacity of approximately 5 meq/100 gm of soil.

Clay particles under 2 microns in size are the major adsorbing and reacting materials in any soil, thus it was decided that

two relatively pure clay types (montmorillonite and kaolinite) would be used in these studies. Six soils were selected for leaching and adsorption studies.

2. Preliminary Study

The first phase of the proposed research concerned the adsorption or exchange ability of selected "pure" montmorillonite and kaolinite clays for the four test chemicals. Hydrazine, UDMH, and pyridine borane are basic in reaction, while nitronium perchlorate forms strong acids on reacting with water. Preliminary work was directed toward hydrazine, UDMH and pyridine borane because it was thought that similar methods of soil analyses could be developed for all three compounds.

The original plan was to adsorb the chemicals on a clay, exchange them from the clay complex with a 1N sodium acetate buffer and then analyze the results. This was first done using UDMH as the test chemical and adsorbing it on a three gram sample of montmorillonite clay. The results were erratic and even 10,000 ppm (v/v) of UDMH gave only 1.5 meq/100 gm of clay. Previous determinations have shown that the "pure" montmorillonite clay has 97 meq/100 gm exchange capacity, yet less than 2 meq/100 gm of UDMH was actually exchanged.

Brindley and Ruston (ref. 3) and Gieseking (ref. 9) have stated that they were unable to remove various organic ions from a clay once the ions were adsorbed. Therefore, it was necessary to determine whether or not the UDMH was adsorbed to the clay particles. This problem was initially approached by checking the concentration of a UDMH solution before and after the addition of a given weight of clay. The difference was tentatively considered to be the amount adsorbed on the clay. However, the method of analysis inevitably includes decomposition and adsorption.

Triplicate montmorillonite clay samples (3 gms) were prepared and treated with 20 ml of a 10 or 100 ppm (v/v) UDMH solution. The mixtures were shaken for 24 hours, centrifuged and the supernatant was analyzed for UDMH. Three identical runs were made and the results, as determined by difference, were compared (Table 2). These results are comparable and suggest that adsorption and/or decomposition occurs on the clay particles.

Although the adsorption was less than 1 meq/100 gm of clay, which is far below the exchange capacity of the montmorillonite clay, a method utilizing the difference between initial and final concentration of test chemical seems workable. This would not

TABLE 2

Adsorption and/or Decomposition of UDMH on a "Pure" Montmorillonite Clay

	Original Concentration (ppm-v/v)	Supernatant Concentration (ppm-v/v)	Retained or Decomposed on Clay (ppm-v/v)	Meq UDMH/ 100 gm Clay*
First Run	100	3.5	96.5	0.84
	10	2.3	7.7	0.067
Second Run	100	17.1	82.9	0.72
	10	1.7	8.3	0.072
Third Run	100	17.7	82.3	0.72
	10	2.4	7.6	0.066

* Meq UDMH/100 gm clay = ppm retained on clay x 0.00873. The millequivalent values for UDMH are equal to millimoles of UDMH. Values given assume no decomposition.

differentiate between adsorption and decomposition but would give a combined value. A high concentration range must be used in order to "saturate" the clay in a reasonable period.

The procedure used in testing for adsorption and/or decomposition of UDMH was developed from these preliminary investigations. This procedure utilized a range of high concentrations in order to saturate the clay in a reasonable period of time.

3. Adsorption and/or Decomposition of UDMH on "Pure" Clays

a. Methods

Duplicate samples of montmorillonite clay (0.2 gms/sample) and kaolinite clay (0.5 gms/sample) were prepared and treated with 500, 1000, 5000 or 10,000 ppm (v/v) solutions of UDMH. Each sample was placed in a 40 ml centrifuge tube. Twenty-five ml of a given test solution was added to the clay sample and the mixture was shaken for two hours. After shaking, the slurry was centrifuged and the UDMH concentration of the supernatant

was obtained and used to determine the quantity of UDMH adsorbed and/or decomposed. The supernatant was decanted and 25 ml of fresh UDMH, at the original concentration, was added. The procedure was repeated at the hours shown in Tables 3 and 4. Five ml of solution remained in the montmorillonite samples and 3 ml remained in the kaolinite samples after decanting the supernatant. This dilution of the fresh 25 ml solution was considered and calculated for each run. As the tables show, longer periods of shaking were used as the clay samples approached the saturation point.

b. Results

The results shown in Tables 3 and 4 need to be discussed in relation to: time required for adsorption and/or decomposition; the difference noted between the two clay types; total adsorption and/or decomposition by both clay types; and, the effect of the UDMH concentrations.

TABLE 3

Effect of Time and Concentration on the Adsorption and/or Decomposition of UDMH (meq/100 gm) on "Pure" Montmorillonite Clay (Averages of Duplicate Samples)

Adsorption Time*	10,000 ppm (v/v)	5,000 ppm (v/v)	1,000 ppm (v/v)	500 ppm (v/v)
2 hrs.	55.5	24.4	8.2	4.9
4 hrs.	97.4	36.3	13.7	8.3
6 hrs.		42.1		11.0
9 hrs.		50.7		14.3
12 hrs.		60.1		17.8
16 hrs.	124.6	63.9	20.3	21.0
22 hrs.	153.7		27.5	
26 hrs.		63.2		25.3
28 hrs.	166.8		32.1	
31 hrs.		76.4		28.0
36 hrs.	158.2		36.3	
40 hrs.		86.1		33.1
50 hrs.	191.8		44.3	
Total meq/100 gms Extracted /	51.6	2.56	3.31	1.37

* At these times old solutions were centrifuged, the supernatant discarded and 25 ml of a fresh solution at the appropriate concentration was added. Where no values are listed in the table, the specific solution was not changed at the indicated time.

/ These values were obtained from Table 5.

TABLE 4

Effect of Time and Concentration on the Adsorption and/or Decomposition of UDMH (meq/100 gm) on "Pure" Kaolinite Clay (Averages of Duplicate Samples)

Adsorption Time*	10,000 ppm (v/v)	5,000 ppm (v/v)	1,000 ppm (v/v)	500 ppm (v/v)
2 hrs.	30.9	11.5	4.9	2.6
4 hrs.	63.8	19.4	9.2	4.1
6 hrs.		18.6		5.6
9 hrs.		22.3		6.7
12 hrs.		25.0		8.2
16 hrs.	78.4	27.4	13.1	9.3
22 hrs.	91.6		20.9	
26 hrs.		29.7		10.4
28 hrs.	95.2		20.9	
31 hrs.		39.8		11.1
36 hrs.	90.4		21.9	
40 hrs.		45.5		13.6
50 hrs.	129.2		25.9	
Total meq/100 gms Extracted /	9.5	0.75	1.32	0.29

* At these times the old solutions were centrifuged, the supernatant discarded and 25 ml of a fresh solution at the appropriate concentration was added. Where no values are listed in the table, the specific solution was not changed at the indicated time.

/ These values were obtained from Table 6.

Time: The time required for adsorption in the case of UDMH is unlike ordinary cation adsorption, if the results are truly indicative of continuing adsorption. According to the USDA Handbook (ref. 19), 15 minutes is all that is required to saturate a clay sample with sodium or ammonium ions. In contrast, it should be noted that after two hours of mixing, less than one-third of the total adsorption of UDMH had occurred on either clay type. It also should be noted that some adsorption was still taking place at the end of 50 hours. Figure 3 shows that the most rapid adsorption occurred during the first four hours. The curves generally indicate a steady rate of adsorption except at several points where there seems to be some desorption. These are possibly points of experimental error. It is possible that the results are more indicative of UDMH decomposition than of UDMH adsorption. Adsorption may be completed within a short period of time and the remaining loss due entirely to decomposition of UDMH.

The long period of continued adsorption, if there is continued adsorption, may be explained by the slightly larger

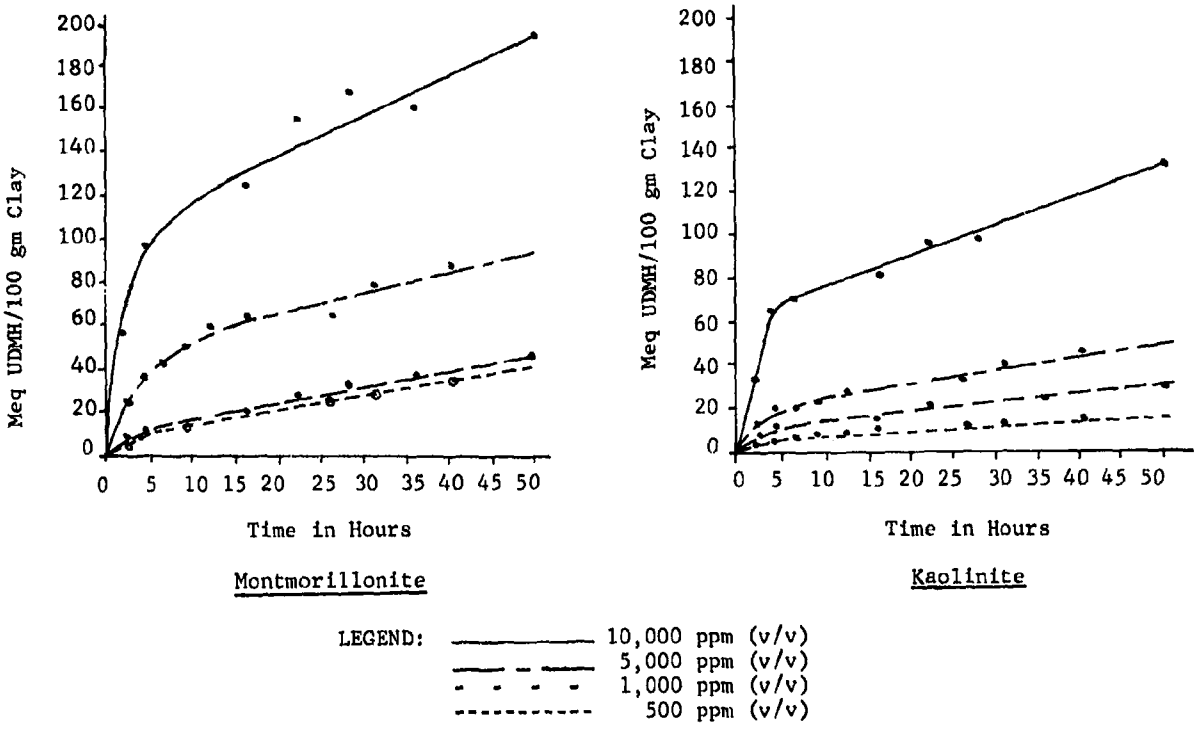


FIGURE 3. Rate of Adsorption and/or Decomposition of UDMH by the Montmorillonite and Kaolinite Clays. Data is Obtained from Tables 3 and 4.

molecular size (UDMH approximately 5Å) (ref. 15), and the stronger organic bonding as compared to inorganic cations. In the case of montmorillonite clay, about 80 percent of the adsorption occurs in the interlayers (within the crystal lattice) of the clay particle. The (001) c-axis spacing varies from 9.6Å (dehydrated) to 15.5Å (2 layers of water molecules) (Appendix C). There is some evidence that the spacing can go as high as 100Å or more; however, at normal spacings, there is a close fit between the size of the UDMH molecule and the available interlayer space. This closeness of fit could explain the length of time required to adsorb the UDMH within the interlayer space. By actual X-ray diffraction analysis (Appendix C), the c-axis spacing of the UDMH saturated montmorillonite clay was 14.98Å, as compared to 15.24Å for water and base saturated clay. This leaves an interlayer spacing of approximately 5.4Å, which compares closely to the 5Å size of the UDMH molecule.

Clay Types: The two types of clay used were almost opposite in their physical characteristics. Montmorillonite has a 2:1 crystal lattice, the ability to swell, a high exchange capacity, a high water holding capacity, a large surface area, adsorption of

cations in the interlayer as well as on the edges, and an abundant isomorphous substitution of atoms within the crystal lattice. Kaolinite has a 1:1 crystal lattice, no ability to swell, a low exchange capacity, a low water holding capacity, a relative low surface area, adsorption only on the edges and surface, and no isomorphous substitution.

The total adsorption and/or decomposition of UDMH reflects these differences between the two clays. Montmorillonite adsorbs and/or decomposes almost twice as much UDMH as the kaolinite at any concentration or time. Thus, the ratio of these two clay types would greatly influence the ability of a particular soil to adsorb and/or decompose UDMH.

Total Adsorption: If the summation values are only adsorption values, then the total adsorption was unusual for both montmorillonite and kaolinite. At the 10,000 ppm level, montmorillonite adsorbed 191.8 meq of UDMH. This is approximately twice the normal cation exchange capacity for montmorillonite. Therefore, much of the apparent adsorption is probably due to the breakdown of UDMH; however, there was definitely some adsorption, as indicated by the 51.6 meq/100 gm of UDMH extracted from the soils at 10,000 ppm concentration as shown in Table 5.

Even though there is considerable breakdown of UDMH in solution and in open containers, there are indications that the UDMH adsorbed on a clay may be somewhat less reactive. Several of the UDMH saturated montmorillonite samples (at 10,000 ppm UDMH) have been preserved for as long as four months in closed bottles. An 18-hour extraction of a sample of these clays with the 1N sodium acetate at pH 5.4 showed the presence of about 1 meq UDMH/100 gm clay.

The adsorption and decomposition of UDMH reported for kaolinite is probably primarily decomposition. Even slight amounts of UDMH that might be adsorbed on the edges of the clay crystal could be readily decomposed. Differential thermal analyses and the X-ray analyses (Appendix C) detected no adsorbed UDMH on kaolinite clay. The 9.5 meq/100 gm of UDMH reported in Table 6 was possibly the result of occluded UDMH molecules in the aggregates that were formed when the clay was washed with alcohol.

Concentration of UDMH: In both montmorillonite and kaolinite, the concentration of UDMH was the controlling factor in determining the rate of adsorption. This is not surprising since cation exchange in the saturation range depends greatly upon the chemical law of mass action.

4. Extraction of UDMH From Montmorillonite and Kaolinite Clays

a. Procedures

The duplicate samples of montmorillonite and kaolinite clay which had been treated with 500, 1000, 5000 and 10,000 ppm UDMH were used in these extraction experiments. These samples were extracted following the 50-hr period of adsorption. Each clay sample was extracted three times with 25 ml portions of 95 percent ethanol. This was followed by eight extractions with 25 ml of 1N sodium acetate buffer (pH 5.4). After each extraction the clay sample was centrifuged and the supernatant was analyzed for extracted UDMH.

b. Results

The rate and amount of UDMH extracted from the treated montmorillonite and kaolinite clays are shown in Tables 5 and 6. The results are given in meq UDMH/100 gm clay. The data presented in these tables indicate that some UDMH is adsorbed by both types of clay. There are some indications that the UDMH extracted from the saturated clays is only part of the total amount retained by the clay. As shown in Table 5, there is a considerable amount of UDMH extracted from the 10,000 ppm level of montmorillonite. However, there is little UDMH extracted from the other concentrations. This may be explained by the findings of Gieseking (ref. 9). Gieseking reported that certain organic molecules were adsorbed in layers. The first layer was found to be very tightly bound, while the succeeding layers could be easily exchanged. If Gieseking's idea of layers is accepted in this case, then it is much easier to explain the data obtained on this basis. At 10,000 ppm the 51.6 meq could represent the second layer of UDMH molecules that are bound with less tenacity. The lower concentrations possibly had not filled the first layer of adsorbed molecules; therefore, it is possible that only a small number of the molecules can be replaced (2 to 4 meq/100 gm) from the first bound layer.

The 9.5 meq of UDMH extracted from the 10,000 ppm saturated kaolinite indicates that van der Waal forces, as well as Coulomb's forces (electro-kinetic) may have been active in such adsorption, if there was any true adsorption on the kaolinite clay.

5. Adsorption and/or Decomposition of Hydrazine on "Pure" Clays

a. Procedure

Duplicate samples of montmorillonite clay (0.2 gms/sample) and kaolinite clay (0.5 gms/sample) were prepared and

TABLE 5

Amounts of UDMH Extracted from the Treated Montmorillonite Clay in meq/100 gm*

Treatment	10,000 ppm (v/v)	5,000 ppm (v/v)	1,000 ppm (v/v)	500 ppm (v/v)
Alcohol				
1	0.0	0.81	0.0	0.46
2	2.01	1.24	1.06	0.91
3	1.45	0.50	0.56	0.0
Sodium Acetate				
1	24.00	0.0	1.63	0.0
2	12.60	0.005	0.06	
3	5.24	0.0		
4	3.07			
5	2.14			
6	0.25			
7	0.49			
8	0.39			
Total UDMH Extracted	51.64	2.555	3.31	1.37

* Clay treatments are discussed in C-3 and the results are shown in Table 3.

TABLE 6

Amounts of UDMH Extracted from the Treated Kaolinite Clay in meq/100 gm*

Treatment	10,000 ppm (v/v)	5,000 ppm (v/v)	1,000 ppm (v/v)	500 ppm (v/v)
Alcohol				
1	0.02	0.0	0.07	0.11
2	0.48	0.41	0.32	0.08
3	0.0	0.32	0.0	
Sodium Acetate				
1	4.32	0.02	0.92	
2	0.88	0.0	0.01	
3	3.07			
4	0.31			0.10
5	0.39			
6	0.03			
7				
8				
Total UDMH Extracted	9.50	0.75	1.32	0.29

* Clay treatments are discussed in C-3 and the results are shown in Table 4.

treated with 500 and 1,000 ppm solutions of hydrazine. Procedures for determining the amount of hydrazine adsorbed and/or decomposed and for extracting the hydrazine from the clay samples are the same as those already outlined for UDMH.

b. Results

The total amount of hydrazine adsorbed and/or decomposed and extracted for each clay type at two hydrazine concentrations are shown in Table 7 and Figure 4. Unlike UDMH, the total adsorption was higher in the more dilute than in the more concentrated solutions. This is in agreement with earlier work on hydrazine (not reported) where there was no adsorption by clay treated with 10,000 or 5,000 ppm solutions of hydrazine, but there was rapid adsorption and/or decomposition at 500 ppm.

At first, it would seem as though the law of mass action is "working in reverse". However, an attempt to explain such findings demands a statement on the physical-chemical nature of colloidal clay.

TABLE 7

Effect of Time and Concentration on the Adsorption and/or Decomposition of Hydrazine (meq/100 gm) on "Pure" Montmorillonite and Kaolinite Clays (Averages of Duplicate Samples)

Adsorption Time*	Montmorillonite		Kaolinite	
	1,000 ppm (v/v)	500 ppm (v/v)	1,000 ppm (v/v)	500 ppm (v/v)
2.5 hrs.		19.6		2.5
3 hrs.	30.0		9.0	
5 hrs.		50.0		
7 hrs.	47.7			
9 hrs.		104.6		7.3
15 hrs.	72.8		12.4	
17 hrs.		187.6		30.3
21.5 hrs.	95.4		14.1	
32.5 hrs.	92.1		11.9	
Total meq Adsorbed and/or Decomposed	92.1	187.6	11.9	30.3
Total meq/100 gms Extracted	0.82	0.25	1.87	1.49

* At these times old solutions were centrifuged, the supernatant discarded and 25 ml of a fresh solution at the appropriate concentration was added. Where no values are listed in the table, the specific solution was not changed at the indicated time.

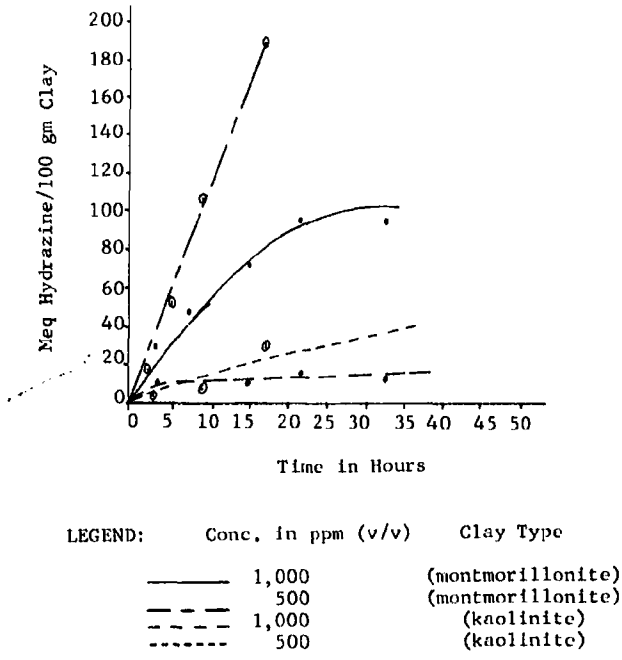


FIGURE 4. Rate of adsorption and/or decomposition of two concentrations of hydrazine by montmorillonite and kaolinite clays.

The negative charged clay particle has a swarm of cations surrounding it. The electrical potential across this layer of cations (Helmholtz double layer) is called the zeta potential, or electro-kinetic potential. As the number of free ions in solution becomes more concentrated, the zeta potential is lowered by a crowding at the double layer (repression effect) until the clay particles are flocculated. It may be that the effective charge and the hydrated size of the hydrazine molecule is of the right magnitude to cause flocculation at 1,000 ppm and above. If this is the case under field conditions where ample soil water is available, it is possible that most of the clay surfaces would be physically unavailable for adsorption of hydrazine at these higher concentrations. The amount retained on the clay would, therefore, be lower.

Flocculation of the clays at high concentrations supports this theory. This phenomenon would have little effect on normal cation exchange where the exchange takes place almost instantaneously. However, where rates of adsorption are slow, flocculation is of primary importance, especially in clay-water systems.

The differential thermal analyses of the hydrazine saturated clays indicated that only small amounts of hydrazine or hydrazine decomposition products were adsorbed. This seems to indicate that the 187 meq/100 gm of hydrazine reported as adsorbed and/or decomposed (Table 7) was largely decomposition. Adsorption was possibly completed within a relatively short period of time.

6. Adsorption of Pyridine Borane on "Pure" Clays

Adsorption of pyridine borane was tested by the procedures worked out for hydrazine and UDMH. When 1,000 ppm pyridine borane solutions were used, there was no adsorption indicated on montmorillonite or kaolinite after 7 hours of shaking. However, conflicting evidence has occurred in the column leaching studies with pyridine borane which places some doubt on the results stated above. Research on the adsorption of pyridine borane by clays needs further work.

7. Adsorption of Nitronium Perchlorate on "Pure" Clays

Nitronium perchlorate forms nitric acid and perchloric acid with the addition of water. The hydrogen ion, associated with the acids, is adsorbed in normal cation exchange. If the concentration of the hydrogen ion is high enough, the effect on the clay particle can be very severe. At a pH of approximately 3 or below, the clay lattice begins to break down. As the clay lattice breaks down under field conditions, there would be drastic changes in the structure of the soil, infiltration of water, permeability, micro-biological activity, exchange capacity, and all of the soil factors that depend upon the identity of the clay crystal. Any concentration of nitronium perchlorate above approximately 100 ppm will have a pH of 3 or below.

Normally, inorganic anions are not adsorbed by clays. Since nitrates and perchlorates are inorganic anions, it would be expected that little or no adsorption of these ions would occur. The clay adsorption studies with 500 ppm nitronium perchlorate indicated no adsorption of nitrates. Perchlorate adsorption was not tested.

8. Adsorption and/or Decomposition of UDMH by Six Different Soil Types

Duplicate samples (0.5 gms per sample) of six soil types (Aiken, Houston, Lufkin, Montezuma, Hanford and Yolo) were prepared and treated with 25 ml of a 5,000 ppm (v/v) water solution of UDMH for 24 hours. The samples were shaken during the 24-hour

adsorption time. After the 24-hour adsorption time, the soil slurries were centrifuged and the UDMH concentration of the supernatant was obtained. The difference between the original and final UDMH concentrations was used to determine the quantity of UDMH adsorbed and/or decomposed.

The adsorption and/or decomposition of UDMH at the end of 24 hours, along with some of the different properties of the clays that were used, are shown in Table 8. Strict cation adsorption would depend primarily on texture, organic matter, and the amount of montmorillonite in each soil. There is little evident correlation between the adsorption and/or decomposition of UDMH and the above factors. However, the amount of nitrates in the soil seems to have an effect on adsorption. Further research is needed to explain the unusual lack of adsorption of the Lufkin soil which is relatively high in montmorillonite clay. Physical conditions, along with chemical aspects not measured, could cause the lack of UDMH adsorption in the Lufkin soil. Since UDMH and hydrazine are reducing agents, the amount of easily reducible materials (nitrates) could effect the breakdown of the material in the soil.

TABLE 8

Comparison of UDMH Adsorption and/or Decomposition at 5,000 ppm (v/v) to Other Physical-Chemical Properties of Six Test Soils

Treatment or Physical-Chemical Properties*	Soil Types					
	Montezuma	Aiken	Yolo	Houston	Hanford	Lufkin
meq UDMH/100 gm (adsorbed and/or decomposed)/	121.0	95.5	79.9	57.9	24.0	0.0
meq UDMH/100 gm (extracted)‡	< 1	< 1	< 0.8	< 1	< 1	< 1
Percent Clay	56	26	28	55	3	50
ppm N as NO_3	8.4	18.3	6.4	< 1	2.4	< 1
Percent Organic Matter	2.75	4.21	2.64	4.74	0.38	1.15
Relative Amount of Montmorillonite	high	undetermined	low	high	none	medium

* Physical-chemical properties were obtained from Table 1.

/ UDMH adsorption and/or decomposition was obtained from duplicate samples run for 24 hours with a single application of UDMH.

‡ UDMH was extracted from the test soils with a 1N sodium acetate buffer (pH 5.4).

Less than 1 meq of UDMH/100 gm soil was extracted from each of the test soils after an alcohol wash and four extractions with 25 ml of 1N sodium acetate buffer (pH 5.4).

9. Leaching Studies With Three of the Test Chemicals

a. Procedures

Soil columns were constructed by connecting five-5 cm diameter by 10 cm long sections of plastic tubing with taped joints. The soil under study was packed in the bottom four sections while the top section was left for the addition of the test solution.

Duplicate columns were run for six soils or soil types (Houston, Lufkin, Montezuma, Hanford, Yolo and a washed sand) using UDMH and pyridine borane at 1,000 ppm (v/v) and hydrazine at 500 ppm (v/v). Eighty ml of the appropriate test solution was placed on the soil column and allowed to leach through. The column sections were disassembled after the test solution had permeated the column and approximately 10 ml of the leachate had been collected. Each 10 cm section of soil was placed in a centrifuge bottle and 100 ml of water was added. The samples were weighed, shaken for 2 minutes and centrifuged. Samples were then taken from the supernatant liquid for analysis. This was followed by oven-drying and weighing of each soil sample. The dilution of the soil was noted, and the concentration of the test compounds in the soil-water was calculated.

b. Results

The results are shown in Tables 9, 10 and 11. Results are given as the percentage of the initial concentration which remains at the indicated soil level. The 0 soil depth is the original solution. The 5, 15, 25 and 35 are averages of each of the 10 cm lengths of soil. The 40 cm value is from the collected leachate. The results indicate some dependence on the amount of clay present in each soil. However, there was little correlation between factors normally associated with adsorption and the amount of test compounds adsorbed and/or decomposed.

The adsorption and/or decomposition of UDMH on small soil samples (Table 8) is comparable with the results of the soil leaching studies (Table 10) with the exception of the Lufkin soil type. The reason for the variation found with the Lufkin soil is unclear. However, it is suggested that under the conditions of the soil leaching study, the Lufkin soil accelerated the decomposition of the UDMH to a greater extent than the other soils.

TABLE 9

Adsorption and/or Decomposition of Hydrazine by Six Different Soils at Several Depths After Leaching of Soil Columns (Expressed as Percent of the Initial Concentration)*

	Soil Type and Leaching Time					
Soil Depth (cm)	Houston 18 hr	Lufkin 30 hr	Montezuma 18 hr	Hanford 3 hr	Yolo 18 hr	Sand 1 hr
0	100.0	100.0	100.0	100.0	100.0	100.0
5	8.0	49.0	23.0	44.0	37.0	100.0
15	0.2	0.6	0.6	72.0	6.0	100.0
25	0.0	0.0	0.0	15.0	0.3	100.0
35	0.0	0.0	0.0	0.6	0.0	100.0
40	0.0	0.0	0.0	2.0	0.0	100.0

* Soil columns were 5 centimeters in diameter by 40 centimeters in length. The results are averages of duplicate column runs using 80 ml of a 500 ppm (on a v/v basis) hydrazine solution (40 mg hydrazine).

TABLE 10

Adsorption and/or Decomposition of UDMH by Six Different Soils at Several Depths After Leaching of Soil Columns (Expressed as Percent of the Initial Concentration)*

	Soil Type and Leaching Time					
Soil Depth (cm)	Houston 18 hr	Lufkin 24 hr	Montezuma 18 hr	Hanford 3 hr	Yolo 18 hr	Sand 2 hr
0	100.0	100.0	100.0	100.0	100.0	100.0
5	60.0	23.0	30.0	41.0	30.0	100.0
15	32.0	0.2	2.0	49.0	6.0	100.0
25	5.0	0.0	0.2	27.0	4.0	100.0
35	3.0	0.0	0.0	3.0	2.0	100.0
40	0.3	0.0	0.0	0.8	1.0	100.0

* Soil columns were 5 centimeters in diameter by 40 centimeters in length. The results are averages of duplicate column runs using 80 ml of a 1,000 ppm (on a v/v basis) UDMH solution (62.4 mg UDMH).

TABLE 11

Adsorption of Pyridine Borane by Six Different Soils at Several Depths After Leaching of Soil Columns (Expressed as Percent of the Initial Concentration)*

	Soil Type and Leaching Time					
Soil Depth (cm)	Houston 24 hr	Lufkin 30 hr	Montezuma 18 hr	Hanford 5 min	Yolo 18 hr	Sand 10 min
0	100.0	100.0	100.0	100.0	100.0	100.0
5	71.0	0.0	23.0	94.0	83.0	100.0
15	18.0	0.0	6.0	77.0	0.2	100.0
25	0.0	0.0	0.2	74.0	0.2	100.0
35	0.0	0.0	0.2	63.0	0.0	100.0
40	0.0	0.0	0.0	45.0	0.0	100.0

* Soil columns were 5 centimeters in diameter by 40 centimeters in length. The results are averages of duplicate column runs using 80 ml of a 1,000 ppm (on a v/v basis) pyridine borane solution (73.6 mg pyridine borane).

The six hour longer leaching time for the Lufkin might be a critical period. The reason for the complete lack of adsorption or decomposition in the small samples of Lufkin soil is not clear.

Pyridine borane was adsorbed in these tests; however, earlier studies indicated that there was no adsorption by "pure" montmorillonite clay at the end of seven hours. The previous tests were conducted in closed bottles. On the other hand, in the leaching studies, there was a source of atmospheric oxygen, organic matter, and other soil contaminants which may have been effective in causing adsorption of pyridine borane, even though no adsorption was noted on the "pure" clays. The presence of oxygen and organic matter may have initiated the breakdown of the pyridine borane but we have no confirming data on breakdown of this compound.

c. Discussion

UDMH, hydrazine, and pyridine borane, in dilute solutions,

can be adsorbed and/or decomposed in a realtively short column of soil if the soil has a moderate amount of clay. From a practical standpoint, there should be little likelihood of contaminating underground water supplies if the above test compounds were spilled on slowly-permeable clay soils. As the texture of the soil grades toward a sand, there would be an increased possibility of contaminating the ground water supplies. Such adsorption on clays would minimize also the dangers of contamination due to run-off water. However, the colloidal clay fraction would be present in large-scale run-off of rainfall waters. These fractions, if they contained high amounts of adsorbed toxicants, could become a problem. However, the problem of adsorption versus decomposition is not as yet sufficiently well understood for our data to be freely interpreted from a true pollution standpoint.

D. Plant Growth and Development

1. Seed Germination

Seed germination tests were set up so the results could be analyzed statistically. The design was a completely randomized block with a 3 x 4 x 5 factorial arrangement of treatments. The design included 3 species of plants (squash, peanut and corn), 4 chemicals (hydrazine, UDMH, pyridine borane and nitronium perchlorate), 5 concentrations (0, 1, 10, 100 and 1,000 ppm), 10 seeds per treatment and 4 replications of each treatment. The technique used was a modified "Roll Towel" technique as proposed by the Committee on Standardized Tests, Association of Official Seed Analysts (ref. 20). The experiment was run using neutralized (pH 6.5) and unneutralized test solutions to determine whether the effect was due to pH, the chemical, or a combination thereof. Only one experiment was done with the pyridine borane since the water solution gave a pH of 6.5 and no neutralization was necessary.

a. Methods and materials

1) Solutions of the four test chemicals were prepared as outlined (B-4). Neutralization was done with HCl or KOH to a standard pH of 6.5. All solutions were prepared just prior to use to minimize loss of the test compounds due to breakdown or vaporization.

2) Seed from the three species used were selected free from visible mechanical and insect damage. Corn seed had been treated with captan, dieldrin and DDT to prevent insect and fungal

damage; squash was treated - treatment unknown; and, peanut was untreated. The seed were soaked in their respective test solutions for 30 minutes prior to being plated out.

3) The "Roll Towel" technique is discussed using a single replicate as an example. A piece of wax paper, twice as large as a regular folded paper towel, was folded in half and a paper towel was placed on top of the wax paper. Then the toweling was moistened with the test solution and 10 seed were placed inside the folded paper towel approximately one inch from the top. The towel, containing the seed, was rolled inside the wax paper and a rubber band was placed around the roll to keep it from unrolling. The towel then served as a wick for supplying the test solution to the seed.

4) Four replicates were used for each treatment. The four replicates of a given treatment were placed in a 4 x 6 inch cylindrical pyrex jar. One hundred ml of the test solution was then placed in the jar and the jar was covered with a plastic cover to reduce volatilization of the test chemicals into the germination chamber. Five 4-mm holes were placed in the plastic cover to allow for O_2 and CO_2 exchange.

5) The covered jars were placed in a forced air germinator which had been precalibrated to maintain $89 \pm 1°F$ within the chamber. A thermometer placed inside a rolled towel at the level of the germinating seed showed a consistent 3-4 degree lower temperature than chamber temperature. Therefore, the seed are considered to have germinated at $86 \pm 2°F$. The humidity at the level of the germinating seed was above 95 percent.

6) Due to the size of the germinator, it was necessary to do the seed germination experiments as a series of experiments. This is not consistent with good statistical design unless the growth conditions are so closely maintained that each experiment in the series has been under exactly the same set of conditions. Since this cannot be completely judged, a single chemical was used on the three seed types at the five concentrations in each experimental run in the above series. Thus the individual experiments can be statistically analyzed by a 3 x 5 factorial analysis or the whole group can be analyzed by a 3 x 4 x 5 factorial analysis.

7) The seed were left in the germinator for 48 hours. Germination counts were taken at 12-hour intervals after the initiation of the experiment. A seed was considered germinated when the radical was 2 mm long, whether inside or outside the seed coat. The first three counts in each test were made by unrolling the

towel and visually estimating the number germinated while the 48-hour reading was taken as an actual measurement. It was necessary to make the visual estimations during the first 3 periods to prevent handling and damage to the germinated seed.

b. Results

A 3 x 4 x 5 factorial analysis was done for the neutralized and unneutralized solutions at each of the four 12-hour observation times. The overall analysis showed significance at the .01 level for treatments, species, concentrations, chemicals and the interaction of these three variables. Significance at the .01 level was found between neutralized and unneutralized solutions and between dates. None of these statistical findings are surprising when the tabular results are carefully analyzed. The statistical results are due to several major effects which were noted at specific times, or because of expected variations between variables.

The major purpose of this experimental approach was to determine the effects of a specific chemical on the over-all ability of three plant species to germinate. The experiment was designed to elicit the greatest amount of information in case inhibition was found at the lower concentrations. None of the chemicals inhibited germination at or below 100 ppm. Thus only the data which best represents the results and purpose of this experiment are presented. This includes the 1 x 3 x 5 factorial analysis for the neutralized 48-hour treatments of each of the four chemicals.

The data obtained at the 48-hour period is quantitative in that an actual measurement was made to determine which seeds fit our definition of germination. The percent germination for each treatment is presented in Table 12. The germination percentages for each species at the five concentrations are graphically presented for each chemical in Figures 5 and 6. The statistical analysis of the tabular information is shown in Table 29 (Appendix D).

The statistical data are rather easily analyzed in these experiments. Hydrazine: significance is shown at the .01 level for treatments, concentration and species - concentration interaction. Observation of the data shows that these significant values lie in the decrease in germination for peanut and corn at the 1,000 ppm concentration level. UDMH: significance is shown at the .05 level for replications and at the .01 level for all other sources of variation. These differences can all be attributed to the great reduction in germination of squash at the 1,000 ppm concentration. Pyridine borane: treatments

TABLE 12

The Effect of Hydrazine, UDMH, Pyridine Borane and Nitronium Perchlorate on the Percentage of Seed Germination*

Treatment	Concentration in ppm †	Squash	Peanut	Corn
Hydrazine	0	82.5	97.5	95.0
	1	92.5	100.0	97.5
	10	87.5	100.0	100.0
	100	85.0	95.0	95.0
	1000	85.0	47.5	62.5
UDMH	0	97.5	97.5	100.0
	1	97.5	97.5	97.5
	10	90.0	97.5	97.5
	100	95.0	97.5	95.0
	1000	50.0	97.5	97.5
Pyridine Borane	0	75.0	100.0	97.5
	1	85.0	100.0	97.5
	10	95.0	100.0	100.0
	100	90.0	100.0	97.5
	1000	85.0	97.5	97.5
Nitronium Perchlorate	0	95.0	97.5	97.5
	1	95.0	95.0	95.0
	10	87.5	100.0	97.5
	100	92.5	100.0	95.0
	1000	90.0	97.5	97.5

* The test solutions were neutralized at pH 6.5. Treatment time was 48 hours at 86 ± 2°F. Forty seed were used per concentration.

† Concentrations are given on a wt/v basis for nitronium perchlorate and on a v/v basis for the other test chemicals.

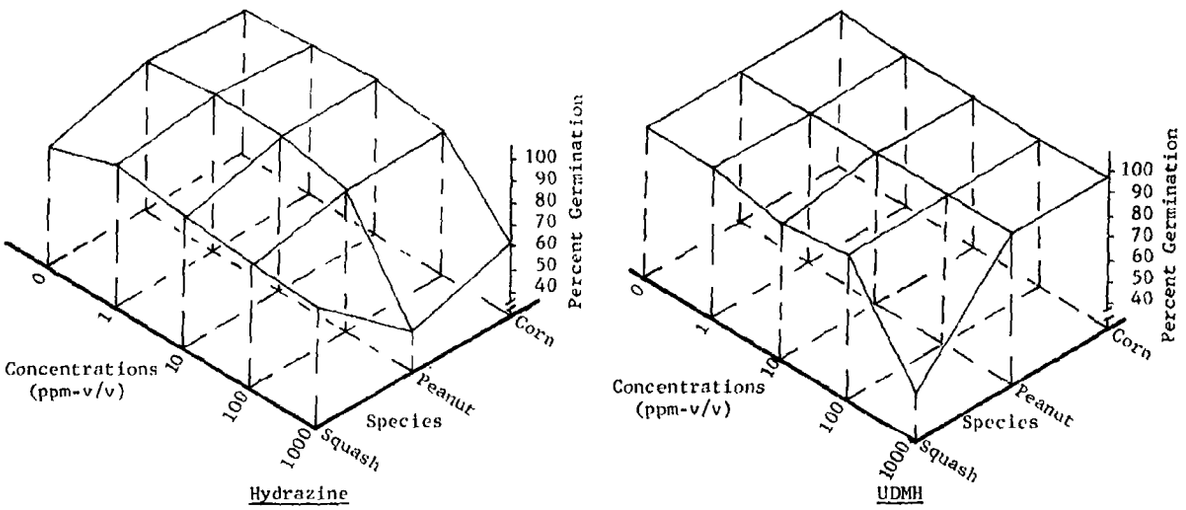


FIGURE 5. Three dimensional graphic representations of the percent germination of three plant species in five concentrations of hydrazine or UDMH. Results are given as concentrations versus plant species versus percent germination.

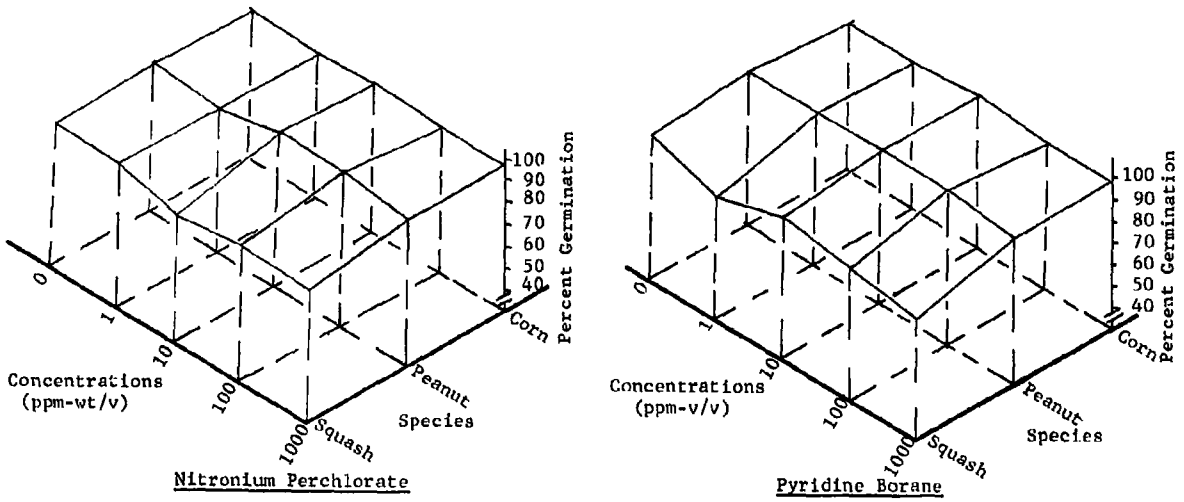


Figure 6. Three dimensional graphic representations of the percent germination of three plant species in five concentrations of nitronium perchlorate or pyridine borane. Results are given as concentrations versus plant species versus percent germination.

and species are significant at the .01 level. Observation of the data shows that this must be due to the decreased germination in most of the squash treatments as compared to peanut and corn. However, there is no concentration effect within the squash. Thus this chemical has no effect on germination even at a concentration of 1,000 ppm. Nitronium perchlorate: the species are significant at the .05 level due again to reduced germination throughout the squash treatments. Thus nitronium perchlorate seems to have no effect on germination per se.

Interpretation of these results from a true toxicity standpoint must of necessity be conditioned on the decomposition rates of hydrazine and UDMH. Until the cause of decomposition is clearly defined, it would be impossible to extrapolate the results to natural conditions.

2. Growth and Development of Seedlings

a. Method and Materials

The germinated seed from the seed germination study were used in these experiments to determine the effects of the test chemicals on seedling growth. Growth in length of the radical (young root) was used as a quantitative measure of chemical toxicity for the peanut and squash. Over-all length of the young plant was used for the corn. Only those seed which germinated were included in the analysis of the effect of the four chemicals on seedling growth.

b. Results

Results are presented in Table 13 and Figures 7 and 8 for the average growth of the germinated seed after 48 hours in neutralized test solutions. The values given are averages from the number of seeds germinated (see Table 12).

TABLE 13

Seedling Growth in Squash, Peanut and Corn Treated with Hydrazine, UDMH, Pyridine Borane and Nitronium Perchlorate*

Treatment	Concentration in ppm /	Squash	Peanut	Corn
Hydrazine	0	32	39	52
	1	34	47	57
	10	22	40	55
	100	23	43	42
	1000	4	9	20
UDMH	0	31	33	49
	1	30	39	54
	10	28	41	49
	100	25	37	49
	1000	10	34	38
Pyridine Borane	0	22	38	44
	1	23	31	44
	10	24	33	49
	100	28	36	49
	1000	17	16	40
Nitronium Perchlorate	0	25	34	40
	1	22	35	39
	10	21	35	42
	100	23	38	47
	1000	18	27	37

* The test solutions were neutralized to pH 6.5. Treatment time was 48 hours at 86 ± 2°F. The values given for squash and peanuts are average lengths in mm of radical growth for the total number of seed germinated out of the 40 seeds in each treatment. The values for corn include both radical and epicotyl growth.

/ Concentrations are given on a wt/v basis for nitronium perchlorate and on a v/v basis for the other test chemicals.

Certain treatments had a pronounced effect on seedling growth as seen in the tabular and graphic presentations. Some of the other treatments show growth which is definitely below the growth of control seedlings.

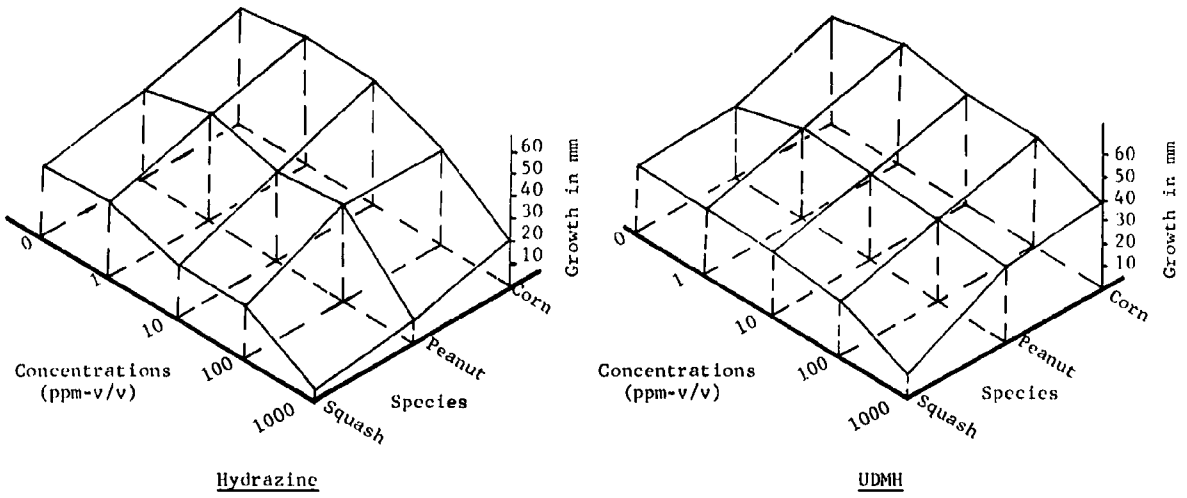


Figure 7. Three dimensional graphic representations of growth in mm of seedlings of three plant species in five concentrations of hydrazine or UDMH. Results are given as concentrations versus plant species versus growth in length of seedlings (mm).

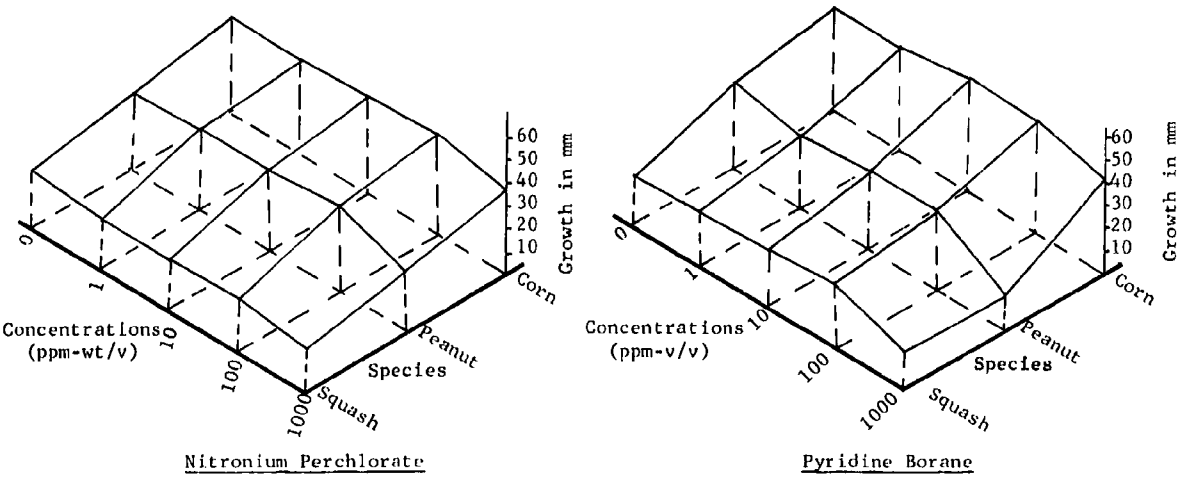


Figure 8. Three dimensional graphic representations of growth in mm of seedlings of three plant species in five concentrations of nitronium perchlorate or pyridine borane. Results are given as concentrations versus plant species versus growth in length of seedlings (mm).

Generally, where inhibition was noted, squash is more sensitive than peanut which is more sensitive than corn.

The chemicals can be rated as to their growth inhibitory effects in decreasing order: hydrazine > UDMH > pyridine borane > nitronium perchlorate.

Results from the first year's study closely parallel those for the present study at 1,000 ppm for hydrazine and UDMH for growth of squash seedlings. There was a greater reduction in the growth of corn seedlings this year than last year at the 1,000 ppm of the two test chemicals. Thus the change in procedure has been worthwhile.

It is still not certain that the procedures used give results which are completely indicative of the potential effect of these chemicals. This is particularly true for hydrazine and UDMH because of their reactivity. First, from our breakdown rates the chemicals are essentially lost within 48 hours - or greatly reduced - thus a constant concentration of the test chemicals was not maintained. Second, the paper toweling served as a wick for supplying moisture and chemical to the test seed. It is possible that the chemicals were adsorbed on the paper toweling and either did not reach the seed or were broken down more rapidly by this adsorption. Since neither hydrazine nor UDMH show appreciable decomposition when prepared in distilled water which is free of trace metal impurities, the extent of decomposition under natural conditions needs to be determined.

3. Young Plant Studies

a. General Methods and Materials

All plants used in these experiments were started in the greenhouse. Seed were planted in 6-inch pots containing the peat-perlite mix (Appendix A). The pots were subirrigated three times a week with a modified full strength Hoagland's nutrient solution (Appendix A) until transferred to the experimental chambers.

b. Water Culture Experiments

1) Methods and Materials

Cotton was used in these experiments since it is a sensitive species and readily lends itself to quantitative growth studies. Cotton was grown in the greenhouse until 10 days (nitronium perchlorate) or 16 days (other chemicals) of age. At this time the pots of cotton were brought into the

laboratory. The plant roots were carefully removed from the peat-perlite mix, washed, and the plants were inserted into the test solutions.

Each growth chamber was used to test a single chemical. Within each experimental chamber five replications containing two cotton plants each were tested at each of seven concentrations. The concentrations used were 0, 10, 50, 100, 300, 600 and 1,000 ppm of chemical in water on a v/v or wt/v basis. Each chamber contained 70 healthy cotton plants at a similar stage of development. Each replicate consisted of a quart jar equipped with a 2-holed styrofoam stopper through which the two test plants were inserted. The plants were grown in a full strength Hoagland's solution plus the appropriate concentration of test chemical. The pH of all solutions was adjusted to 6.5. Plants were grown under these conditions for 9 days with a single nutrient solution change after 5 days of treatment. These cultures were unaerated. Environmental conditions for the test chambers are listed under B-7.

2) Results

Quantitative growth data was obtained for leaf size, plant height and leaf number. The rate of growth during the experimental treatment was too slow for reliable quantitative data and the results of these measurements were not summarized.

Qualitative indices of plant injury or death were noted on a 0-8 basis, with 0 as no injury and 8 as plant death. The results of these observations are included in Table 14.

Earlier experiments have shown that both hydrazine and UDMH decompose, or are lost in some way, with time. This may be the reason why little or no injury was noted in the plants maintained in cultures at a low concentration of both of these chemicals.

The culture jars in this series of tests were not covered to exclude light from the nutrient solution. Thus all control jars developed a healthy algal growth - probably Chlorella. No algal growth was seen at any concentration of hydrazine or pyridine borane. Some algal growth was noted at the 10 ppm concentrations of UDMH and nitronium perchlorate but the growth was not as pronounced in any of these replications as it was in the control replicates. An indication of algal development was noted in all the replicates at 50 ppm of UDMH and in one of the replicates at 50 ppm of nitronium perchlorate. None of the higher

TABLE 14

Injury Index of Four Test Chemicals on Cotton Seedlings Grown in Water Culture

Chemicals	Treatment Time (Days)	Concentrations in ppm*						
		0	10	50	100	300	600	1000
Hydrazine	1	0	0	0	2	4	7	7
	4	0	0	0	2	8	8	8
	9	0	0	4	5	8	8	8
UDMH	1	0	0	0	0	0	0	0
	4	0	0	0	0	4	6	8
	9	0	0	3	3	5	8	8
Pyridine Borane	1	0	0	0	0	0	2	4
	4	0	2	2	4	8	8	8
	9	0	2	6	8	8	8	8
Nitronium Perchlorate	1	0	0	0	0	0	0	0
	4	0	0	2	2	2	2	2
	9	0	0	2	4	5	6	7

* On a wt/v basis for nitronium perchlorate and a v/v basis for the other test chemicals.

concentrations of these two chemicals showed any algal growth. These observations are in accord with preliminary algal studies undertaken in the aquatic phase of this research.

A descriptive discussion of the effects of each chemical on cotton should be of value. Hydrazine: The first symptom, which occurs within 24 hours in the 300, 600 and 1,000 ppm solutions is a dehydration of all foliage without chlorosis or necrosis. Death occurs at the higher concentrations within 30 hours and at 300 ppm in 48 hours. Cotyledon injury at the lower concentrations shows chlorosis and necrosis starting at the margin and becoming interveinal, followed by abscission or death. Initial injury in the leaves, at the lower concentrations, shows vein clearing and browning with some epinasty of petioles. Where injury was noted the petioles seem to dehydrate midway between the leaf blade and stem. The conducting tissues remain intact since the leaf blade remains green or partially green even after petiole collapse. UDMH: Early symptoms show a general flacidity of the leaves followed by dehydration with death occurring in 48 hours at 1,000 ppm. The cotyledons and true

leaves become necrotic without chlorosis. Leaf necrosis occurs along the main veins of the leaf and gradually becomes interveinal. Some leaves exhibited localized marginal lesions. Pyridine borane: The first symptoms are the same as described for hydrazine with death occurring at a similar rate. An additional symptom is the reddening and enlarging of the gossypols which increase in size and intensity of color with increasing concentrations from 10 through 1,000 ppm. Early symptoms at the lower concentrations show a general flaccid appearance of the plants. The upper stem and petiole tissues are killed first, thus eventually causing top death. The intensity of the response is directly proportional to concentration of test chemical. Nitronium perchlorate: Young leaves show puckering and incurling with some marginal and interveinal necrosis. These conditions are somewhat localized even at the highest concentration. Stem death occurs at concentrations above 300 ppm, but this stem death had not caused plant death at the termination of the experiment.

c. Spray Experiments

Preliminary experiments, where plants were sprayed to the drip point with a specific concentration of chemical, showed that sprays of the four test chemicals were not highly effective in causing plant injury, except at high concentrations and then only if a wetting agent was applied with the spray. A 4,000 ppm water solution of each chemical sprayed to the drip point on each of six test species gave only a slight temporary injury to three of the test plants. A 10,000 ppm spray with an added wetting agent produced injury in all test plants with each chemical. In some cases the plants were killed.

Based on these preliminary experiments the following spray study was initiated and the results carefully catalogued.

1) Methods and Materials

Six plants (5 species) were grown, 2 plants per pot, in the peat-perlite mix. These plants were: cotton - 25 days old, cotton - 13 days old, pinto bean - 13 days old, soybean - 25 days old, endive - 13 days old, and squash - 12 days old. One pot of each species was sprayed with 2,000, 6,000 or 10,000 ppm of each test chemical and another pot was held for control comparisons. Each treatment was done with a neutralized and an unneutralized spray. Each treatment was made up in 100 ml of distilled water to which was added 0.1 ml of a 0.1% multifilm spreader activator X-77.

The six pots constituting a treatment were placed in a tray and sprayed with the appropriate spray treatment until the spray started to drip from the plants. The treated and control plants were then placed in the greenhouse and observed daily for injury symptoms.

2) Results

A qualitative injury index (on a 0-8 basis) was used as with the water culture experiments. These results are presented in Tables 15, 16, 17 and 18 for all the treatments. Injury is shown three hours after the spray application, at the end of one day and after a five-day period when partially injured plants had started to recover.

TABLE 15

Injury Index for Hydrazine, When Applied As a Spray to Plants

Plant	Time After Application	Concentrations (ppm v/v)			
		0	2,000	6,000	10,000
Endive	3 hrs.	0	2	3	7
	1 day	0	4	7	8
	5 days	0	4	8	8
Soybean	3 hrs.	0	2	5	7
	1 day	0	2	5	8
	5 days	0	2	8	8
Pinto bean	3 hrs.	0	2	3	7
	1 day	0	3	5	8
	5 days	0	3	8	8
Squash	3 hrs.	0	2	3	4
	1 day	0	3	4	6
	5 days	0	3	8	8
Cotton	3 hrs.	0	1	2	4
	1 day	0	3	3	4
	5 days	0	3	3	4

TABLE 16

Injury Index for UDMH, When Applied As a Spray to Plants

Plant	Time After Application	Concentrations (ppm-v/v)			
		0	2,000	6,000	10,000
Endive	3 hrs.	0	0	0	0
	1 day	0	0	2	3
	5 days	0	0	3	4
Soybean	3 hrs.	0	2	3	3
	1 day	0	2	3	3
	5 days	0	2	4	4
Pinto bean	3 hrs.	0	2	2	2
	1 day	0	2	2	2
	5 days	0	2	2	2
Squash	3 hrs.	0	0	0	0
	1 day	0	1	2	2
	5 days	0	2	2	2
Cotton	3 hrs.	0	0	0	3
	1 day	0	0	0	3
	5 days	0	0	0	3

TABLE 17

Injury Index for Pyridine Borane, When Applied As a Spray to Plants

Plant	Time After Application	Concentrations (ppm-v/v)			
		0	2,000	6,000	10,000
Endive	3 hrs.	0	0	0	4
	1 day	0	0	0	4
	5 days	0	0	1	8
Soybean	3 hrs.	0	2	2	4
	1 day	0	2	2	4
	5 days	0	2	2	4
Pinto bean	3 hrs.	0	2	2	4
	1 day	0	2	2	4
	5 days	0	2	2	4
Squash	3 hrs.	0	0	1	2
	1 day	0	0	1	3
	5 days	0	0	2	4
Cotton	3 hrs.	0	1	2	2
	1 day	0	1	2	4
	5 days	0	1	2	4

TABLE 18

Injury Index for Nitronium Perchlorate, When Applied As a Spray to Plants

Plant	Time After Application	Concentrations (ppm-wt/v)			
		0	2,000	6,000	10,000
Endive	3 hrs.	0	0	4/0*	6/2
	1 day	0	0	4/1	6/3
	5 days	0	1	4/1	8/3
Soybean	3 hrs.	0	2	4	4
	1 day	0	2	5	5
	5 days	0	2	5	5
Pinto bean	3 hrs.	0	1	2	3
	1 day	0	1	3	4
	5 days	0	1	4	4
Squash	3 hrs.	0	0	2	3
	1 day	0	0	2	4
	5 days	0	1	2	4
Cotton	3 hrs.	0	1	2	3
	1 day	0	1	3	4
	5 days	0	2	3	4

* The unneutralized solution, in the case of endive, gave marked effects which were not seen in the neutralized. Thus for 6,000 and 10,000 ppm both values are given as unneutralized/neutralized.

The initial effect noted in all affected plants at all concentrations was a wilting followed by a flaccid water-soaked appearance of the leaves. This latter response was local in nature or a whole plant response depending upon the severity of final injury. A descriptive discussion of the effects of each chemical on the group of plants is presented. The severity of the response depended upon toxicant concentration and plant species concerned. Hydrazine: Leaves becoming wilted and flaccid with a water-soaked appearance. This was followed by edge necrosis with leaf curl or scattered necrotic spotting. Where entire foliage became severely water-soaked the necrotic spotting spread rapidly to include whole leaves and the whole plant. UDMH: Effects, where noted, followed those outlined for hydrazine. Several species showed no effects after three hours or only slight wilting. Where injury does occur, it develops within the first day and occurs as scattered necrotic spotting with occasional leaf curl. Pyridine borane: Symptoms are similar to those noted in hydrazine. The severity of symptom occurrence is between that

for UDMH and nitronium perchlorate. Nitronium perchlorate: Symptom development is identical with that for hydrazine but plant injury was generally not as widespread or severe as with the hydrazine.

Plant injury, in most cases, was completely developed or almost completely developed within the first three hours after treatment. Where plants became wilted with only slight necrotic spotting, the wilted appearance disappeared within the first day. At the end of the five-day observation period, the plants, which were not killed, were all recovering. Recovery was noted except in necrotic areas. Growth could be correlated with the amount of tissue damaged.

Severity of response to the three concentrations of the four test chemicals by the five plant species showed: endive > soybean > pinto bean > squash > cotton.

There was no evident difference in the severity of response when neutralized spray solutions were used instead of unneutralized solutions. Where slight differences occurred, the values have been averaged so the results shown in Tables 18 - 21 are essentially average results from two replicated experimental test series.

d. Fumigation Experiments

These experiments were designed to see if any one of the four test chemicals would produce injury in various species of plants when the chemicals were added as air pollutants or fumigants.

1) General Methods

Seven species of plants were used in these experiments: soybean, cowpea, pinto bean, cotton, endive, alfalfa and squash. The plants were grown in the greenhouse in standard peat-perlite mix for 16-21 days. Fumigations were carried out with each test chemical at three different concentrations. Thus only one chemical was tested at one time. The first growth chamber was used as a control chamber and the other three were the experimental chambers. Each chamber contained two pots with two plants per pot for each of the seven plant species - with the exception of endive and alfalfa where an indefinite number of plants was used per pot. The concentrations of the test chemicals varied greatly depending upon the vapor pressure of the specific chemical. These concentrations are discussed under fumigation methods.

The plants were fumigated for from 3 to 24 hours and then observed over a six-day period for evidence of plant injury.

2) Methods of Fumigation

Methods for measuring and injecting gases as air pollutants or air fumigants have been detailed in an earlier report (ref. 12). However, the introduction of a liquid into the atmosphere at a known concentration requires different techniques. Air flow measurements were obtained by use of the calibrated orifice meters on the chamber outlet ducts. These measurements gave an air flow of 450 to 600 liters per minute depending on the chamber in use.

Calculations of vapor concentrations in the test chambers is dependent upon the vapor pressure of the liquid at the dispensing temperature. Usually these vapor pressures are given at 25°C and atmospheric pressures. Knowing the air flow in liters per minute, the value for the number of cc of vapor necessary to give a specific chamber concentration on a v/v basis (vapor/air) can be calculated from the following equation:

$$\text{cc of vapor} = \text{ppm} \times \text{liters of air per minute} \times 10^{-3}$$

For example, in chamber 3, 50 ppm of hydrazine was used in an air flow of 500 L/min, which required:

$$50 \times 500 \times 10^{-3} = 25 \text{ cc of hydrazine vapor.}$$

In generating hydrazine vapor N_2 gas was passed through liquid hydrazine which was held at 30°C. The vapor pressure of hydrazine at 30°C is 19.3 mm of mercury. The following equation was used to calculate the N_2 flow required to maintain a given level of hydrazine vapor in the test chambers:

$$\frac{N + L}{L} = \frac{P}{VP}$$

Where N is the N_2 flow, L is the cc of liquid vapor, P is atmospheric pressure, and VP is the partial pressure of the test liquid at a given temperature. Thus hydrazine at 50 ppm requires:

$$\frac{N + 25}{25} = \frac{760}{19.3} \; ; \; N = 960 \text{ cc/min of } N_2.$$

These equations were used in calculating the nominal chamber concentrations of liquid vapor.

Apparatus for injection of the test liquids was designed so all liquids could be dispensed into the test chambers in a similar fashion. The liquid to be vaporized was placed in an 18 inch x 1 1/2 inch diameter pyrex tube with a 24/40 female joint. A medium porosity gas dispersion tube was attached to a gas inlet-outlet adapter with a 24/40 male joint and was inserted into the pyrex tube containing the test liquid. Two of these were connected in series and kept submerged in a constant temperature water bath. The bath containing the pyrex tubes was enclosed in a plastic hood to insure a constant temperature around the upper end of the tubes. The second tube in the series was attached to a small overflow flask. From this the test vapor passed directly into the test chamber.

The overflow flask and all connecting tubing was wrapped with heating tape and kept at 5°C above the temperature of the water bath. Water pumped nitrogen was metered from a large tank of nitrogen equipped with a two-stage regulator. The nitrogen passed through a soap film flow tube for calibration purposes, through a drying tube to remove the water, past an open ended mercury manometer to measure back pressure, through the first then second gas dispersion tubes, through heated lines to the overflow flask (which was also heated), through heated lines to the test chamber, and injected in the test chamber over a small fan which immediately mixed the test vapor with the chamber air. Three of the above set-ups were operated simultaneously to produce a different concentration of the test chemical in each of the three experimental chambers. A separate series of experiments was run for each test chemical.

The flow rates of the N_2 carrier gas in both pyridine borane and perchloric acid were limited due to the frothing of the liquids as N_2 was dispersed through them. Higher flows would have carried these liquids into the chambers.

3) Results

Under the conditions of the fumigation procedures none of the plants were injured at any concentration of perchloric acid or pyridine borane. These test chemicals were used at very low chamber concentrations for 24-27 hours.

Actual chamber concentrations of both hydrazine and UDMH, although below the nominal concentrations, were sufficiently high that plant injury was initiated within a short period of

time. Thus hydrazine fumigations were carried out for 3-4 hours and UDMH fumigations for 4 hours. Plant injury, unlike injury in the spray studies, continued to develop after the fumigation was stopped. Intensity of plant injury was almost completed within 24 hours from the start of the fumigation period.

Injury noted with hydrazine and UDMH varied somewhat from plant to plant but the overall effects on the seven test species were very similar for the two test chemicals (Tables 19 and 20). Symptom development was also characteristic for the two chemicals. The initial symptom noted with all plants was a general wilting of the leaves and then the whole plant. Wilting was followed by a water-soaked appearance of interveinal leaf areas. Associated with this was an incurling of leaf margins. Where the water-soaked appearance was noted, it was followed by necrotic spotting and leaf death in severe cases.

TABLE 19

Injury Index for Hydrazine When Applied As a Fumigant to Plants*

Plant	Time After Fumigation	Nominal Concentrations (ppm-v/v)			
		0	25	50	75
Soybean	2 hrs.	0	2	5	5
	1 day	0	3	6	6
	6 days	0	6	8	8
Cowpea	2 hrs.	0	5	6	6
	1 day	0	5	6	6
	6 days	0	5	7	7
Pinto bean	2 hrs.	0	3	6	6
	1 day	0	4	6	7
	6 days	0	8	8	8
Cotton	2 hrs.	0	0	0	5
	1 day	0	2	5	6
	6 days	0	4	6	7
Endive	2 hrs.	0	0	0	0
	1 day	0	4	6	6
	6 days	0	8	8	8
Alfalfa	2 hrs.	0	1	2	3
	1 day	0	3	6	6
	6 days	0	5	8	8
Squash	2 hrs.	0	1	2	3
	1 day	0	2	5	5
	6 days	0	2	7	7

* Plants were fumigated at the indicated concentrations for 4 hours.

TABLE 20

Injury Index for UDMH When Applied As a Fumigant to Plants*

Plant	Time After Fumigation	Nominal Concentrations (ppm-v/v)			
		0	31	62	94
Soybean	0 hrs.	0	3	5	6
	1 day	0	5	6	6
	6 days	0	7	7	7
Cowpea	0 hrs.	0	0	2	4
	1 day	0	5	5	7
	6 days	0	5	6	7
Pinto bean	0 hrs.	0	0	1	3
	1 day	0	3	4	5
	6 days	0	3	6	7
Cotton	0 hrs.	0	1	5	5
	1 day	0	6	7	7
	6 days	0	6	7	7
Endive	0 hrs.	0	1	2	2
	1 day	0	7	7	8
	6 days	0	8	8	8
Alfalfa	0 hrs.	0	0	1	2
	1 day	0	6	7	7
	6 days	0	7	8	8
Squash	0 hrs.	0	1	3	3
	1 day	0	3	5	5
	6 days	0	5	6	6

* Plants were fumigated at the indicated concentrations for 3-4 hours.

Plant death in the hydrazine fumigations included soybean, pinto bean, endive and alfalfa, while with UDMH only endive and alfalfa were killed. At the end of six days all plants that were not dead were starting to recover.

4. Discussion

A discussion of the effects of the four test chemicals used in these experiments on various species of plants should be divided into two areas. The effects noted with hydrazine and UDMH are somewhat comparable and will be discussed together as will the results with pyridine borane and nitronium perchlorate.

Pyridine borane and nitronium perchlorate did not affect seed germination in the three species tested at any concentration. Seedling growth was affected only at the 1,000 ppm concentration and even at this concentration corn was not injured. Water culture studies showed severe injury or death only at the higher concentrations. Spray applications at 2,000 and 6,000 ppm did

not severely injure any of the test species. Even the 10,000 ppm spray produced severe injury in only one plant, endive. Fumigation treatments were ineffective due to the vapor pressure of the test chemicals. From the experimental results presented it is not felt that these two chemicals would be important environmental contaminants to plants. The vapor pressure is such that even large spills should not cause air pollution problems. If heavy spills were well washed down with water, these chemicals should not cause plant injury at any distance from the area of spill itself. The soils should neutralize the acids of nitronium perchlorate breakdown and pyridine borane should not percolate rapidly into the soils due to its insolubility. The anionic nature of the nitric and perchloric acids should preclude a buildup in the soils over a long period and the leaching should be sufficiently slow that no toxic concentrations would be expected in the ground waters. This should also hold true for pyridine borane. This chemical is weakly cationic and should be leached much more slowly than the acids but this compound should slowly decompose under natural conditions.

Hydrazine and UDMH are much more toxic than the above two chemicals. However, even these chemicals were not especially toxic at the lower concentrations for seed germination, seedling growth, water culture or spray studies. Hydrazine caused a slight reduction in the growth of squash seedlings at 10 ppm. The fumigation experiments produced severe toxicity at 25-30 ppm for both hydrazine and UDMH. From the plant studies which have been completed, we do not feel either hydrazine or UDMH would act as general environmental contaminants to plants per se, provided both chemicals break down as rapidly under natural conditions as they did under the conditions of these experiments. Large spills would cause severe plant injury in the area of the spill. With a little wind many plants downwind from a heavy hydrazine or UDMH spill would be severely injured or killed. Large spills going into the soil would tend to adhere to the clay fractions, as cation exchange components, provided they were sufficiently diluted with water. Thus very slow leaching would be expected.

Until the cause of decomposition is clearly defined, it would be impossible to extrapolate the results to natural conditions.

E. Aquatic Life

1. Methods and Results

a. Fish

The fish were kept in a holding aquarium without food for at least two days before use. Methods followed closely those used in an earlier report (ref. 12). The test solutions were made up in 6 liter lots in glass jars of either 10 or 20 liter capacity. The two types of jars had the same diameter so there was no variation in the volume-surface area relationship. The fish were added to the freshly prepared test solutions and unless otherwise indicated aeration was begun with a standard aquarium aeration stone. Standard reference water pH was 8.2 unless otherwise indicated. The pH readings were not routinely taken. Preliminary measurements showed that the pH values for the concentrations used were within the range at which no pH effect had been observed in past experiments (pH of 4.5 to 9.0). Where pH, as such, may be a factor, the data are given. Death of the fish was used as the test end point. The fish were considered to be dead when they had stopped moving and did not respond to touch from a probe.

Length-weight data from representative lots of fish are given in Table 21. The aquatic test series were not replicated.

1) TLm Series

The majority of the experiments were aimed at calculation of the median tolerance limit (TLm) which is that concentration at which half of the test organisms remain alive for the time specified. Tentative TLm values were calculated for 24 and 48 hours wherever the data were adequate by the graphical interpolation method given by Duodoroff (ref. 8). The data from the experiments is presented in Tables 22, 23, 24 and 25. The tentative TLm values for hydrazine, UDMH and pyridine borane are summarized in Table 26.

2) The Effect of the Age of the Test Solution on Toxicity

A series of bio-assay experiments was run with goldfish to supplement the analytical studies on the breakdown of the test chemicals in the standard reference water.

TABLE 21

Length-Weight Data From Representative Lots of Fish

Species	No. of Fish	Length in mm		Weight in Grams	
		Average	Range	Average	Range
Goldfish	20	66.5	52-92	4.1	1.8-8.5
Green sunfish	10	60.3	53-68	6.9	5.2-9.7
Bluegill (small)	52	25.8	21-41	0.58	
Bluegill (large)	22	65.6	48-94	10.8	3.1-29.6
Channel catfish	20	61.0	51-71	3.8	1.8-5.7
Largemouth bass	25	59.4	46-91	5.3	

TABLE 22

The Toxicity of Hydrazine to Selected Aquatic Organisms[a]

Species	Concentration (ppm-v/v)	Number of Organisms Alive at Indicated Hours			
		Start	1	24	48
Daphnia pulex	0	12	12	8	
	1	10	10	6	
	1.8	10	10	1	
	3.2	11	11	1	
	5.6	11	11	0	
	10	10	10	0	
Goldfish	0	10	10	9	9
	1.0	10	10	9	9
	2.5	10	10	7	6
	5.2	10	10	5	2
	7.5	10	10	0	0
	10	10	10	0	0
Green Sunfish	0	10	10	10	9
	1	9	9	9	9
	1.8	9	9	9	8
	3.2	9	9	9	9
	5.6	8	8	2	6
	10	9	9	0	0
Bluegill (small)	0	10	10	9	9
	1.0	10	10	6	5
	2.5	10	10	5	5
	5.2	10	10	4	4
	7.5	10	10	5	5
Bluegill (large)	0	5	5	5	5
	2.5	5	5	5	5
	5.2	5	5	5	5
	7.5	5	5	5	0
Channel Catfish	0	10	10	10	10
	1.0	10	10	7	7
	2.5	10	10	0	0
	5.2	10	10	0	0
	7.5	10	10	0	0
	10	7	7	0	0
Largemouth Bass	0	9	9	9	9
	1.0	9	9	9	9
	2.5	9	9	9	9
	5.2	9	9	3	3
	7.5	8	8	0	0
	10	8	8	0	0

[a] Daphnia were tested in chlorine free tap water and the fish species in the standard reference water at a pH of 8.2. The pH of the 10 ppm hydrazine solutions was 8.5.

TABLE 23

The Toxicity of DDBH to Selected Aquatic Organisms*

Species	Concentration (ppm-v/v)	Number of Organisms Alive at Indicated Hours			
		Start	1	24	48
Daphnia pulex	0	11	[illegible]	10	
	10	11	[illegible]	11	
	18	10	[illegible]	7	
	32	10	[illegible]	5	
	56	10	[illegible]	[illegible]	
	100	10	[illegible]	[illegible]	
Goldfish	0	10	10	10	10
	13.5	10	10	10	10
	24	10	10	10	10
	42	10	10	10	10
	75	10	10	10	1
Goldfish#	0	10	10	10	10
	13.5	10	10	10	10
	24	10	9	9	9
	42	10	8	8	8
	75	10	10	9	8
Green Sunfish	0	10	10	10	10
	24	10	10	10	5
	42	10	10	10	0
	75	10	10	5	0
	100	10	10	1	0
Bluegill (small)	0	10	10	9	9
	13	10	10	10	8
	24	11	11	6	0
	42	10	10	2	0
	75	10	10	0	0
Bluegill (large)	0	5	5	5	5
	13	5	5	5	5
	24	5	5	4	0
	42	5	5	0	0
	75	5	3	0	0
Channel Catfish	0	10	10	10	
	5.6	10	10	1	
	10	10	10	0	
	13	10	10	0	
	24	10	10	0	
	42	10	10	0	
Largemouth Bass	0	10	10	10	
	5.6	10	10	10	
	10	10	10	6	
	13	9	9	8	
	24	10	10	10	
	42	8	8	0	

* Daphnia were tested in chlorine free tap water and the fish species in the standard reference water at a pH of 8.2. The pH of the 75 ppm DDBH solutions was 8.6.

All concentrations were adjusted to pH 6.4.

TABLE 24

The Toxicity of Pyridine Borane to Selected Aquatic Organisms*

Species	Concentration (ppm-v/v)	Number of Organisms Alive at Indicated Hours			
		Start	1	24	48
Daphnia pulex	0	11	11	10	
	3.2	14	14	10	
	5.6	10	10	9	
	10	10	10	10	
	18	10	10	8	
	32	10	10	7	
Goldfish	0	5	5	5	5
	1	5	5	5	5
	10	5	5	5	0
	100	5	5	0	0
Goldfish	0	10	10	10	10
	1.8	10	10	10	10
	3.2	10	10	10	10
	5.6	10	10	10	10
	10	7	7	7	6
Goldfish (12 liters unaerated)	0	8	8	8	8
	5.6	8	8	8	8
	10	8	8	8	5
	18	8	8	6	2
	32	8	8	4	0
Green Sunfish	0	10	10	10	10
	3.2	10	10	10	10
	5.6	10	10	10	10
	10	10	10	10	10
	18	10	10	10	5
Channel Catfish	0	10	10	10	9
	5.6	10	10	10	9
	10	10	10	10	0
	18	10	10	3	0
	32	10	10	0	0
	56	10	10	0	0
Largemouth Bass	0	10	10	10	
	5.6	8	8	6	
	10	9	9	5	
	18	8	8	5	
	32	10	10	0	
	56	10	10	0	

* Daphnia were tested in chlorine free tap water and the fish species in the standard reference water at a pH of 8.2.

TABLE 25

The Toxicity of Nitronium Perchlorate to Selected Aquatic Organisms*

Species	Concentration (ppm-wt/v)	Solution pH	Number of Organisms Alive at Indicated Hours			
			Start	1	24	48
Daphnia pulex	0		10	10	10	
	10		10	10	13/	
	18		11	11	10	
	32		12	12	10	
	56		11	11	9	
	100		10	10	3	
Goldfish	0	8.2	10	10	10	10
	24		10	10	10	10
	42		10	10	10	10
	75		10	10	10	10
	100	4.8	10	10	10	10
Green Sunfish	0	8.2	10	10	10	10
	24		10	10	10	10
	42		10	10	10	10
	75		10	10	10	10
	100	4.8	10	10	10	10
	200	3.1	7	7	0	0
	200	8.9	8	8	8	8
	400	7.0	10	10	10	9
	800	7.0	10	10	10	9

* Daphnia were tested in chlorine free tap water and the fish species in the standard reference water.

/ Test organism reproduced during course of experiment.

TABLE 26

Tentative TLm Values for Selected Aquatic Organisms (ppm-v/v)

Organism	Hydrazine		UDMH		Pyridine	Borane
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Daphnia pulex	1.15		38		> 32	
Goldfish						
Aerated	4.2	2.8	> 75	58	32	3.2
Unaerated					32	12.5
Green Sunfish	5.5	5.1	75	24	> 18	18
Bluegill (small)	2.4-7.5		26	16.5		
Bluegill (large)	5.2	5.2	30	18		
Channel Catfish	1.6	1.6	< 5.6		15.5	7.3
Largemouth Bass	3.6	3.6	32		18	

Fifty liter lots of hydrazine, UDMH and pyridine borane were prepared in the standard reference water at concentrations above the TLm values. A single 6 liter aliquot was withdrawn from each lot immediately after preparation and the experimental fish were then added. This process was repeated for each chemical at 24, 48, and 72 hours after the original preparation of the solution. Mortality was followed in each jar for 96 hours. The data from these tests are presented in Table 27.

TABLE 27

The Toxicity of Aged Test Solutions to Goldfish*

Chemical (Initial Concentration in ppm - v/v)	Age of Test Solutions in Hours/	Number of Fish Alive at Indicated Hours				
		Start	24	48	72	96
Hydrazine (7.5)	0	10	2	0	0	0
	24	10	10	10	10	10
	48	10	10	10	10	10
	72	10	10	10	10	10
Control		10	10	10	10	10
UDMH (100)	0	10	9	8	4	1
	24	10	9	6	4	0
	48	10	9	6	3	1
	72	10	10	10	10	10
Control		10	10	10	10	10
Pyridine Borane (56)	0	10	1	0	0	0
	24	10	0	0	0	0
	48	10	0	0	0	0
	72	10	2	0	0	0
Control		10	10	10	10	10

* The goldfish were tested in the standard reference water.

/ Fish were added to the test solutions after the indicated aging period.

3) Timed Exposure Tests

A series of tests was run to obtain data on the speed with which a lethal dose of the chemical would be taken in by the fish. Solutions of hydrazine, UDMH and pyridine borane were prepared at well above the TLm levels. Fish, in plastic mesh bags, were dipped into these solutions for specified periods of time. The fish were then washed and placed in 6 liters of fresh reference water. Data on these experiments are given in Table 28.

TABLE 28

Short Exposure Toxicity of Test Chemicals to Fish

Chemical and Concentration/	Organism	Exposure Time* (Minutes)	Number of Fish Alive at Indicated Hours			
			Start	1	24	48
Hydrazine (100 ppm)	Goldfish	0	10	10	10	10
		1	10	9	9	8
		10	10	10	10	10
		60	10	0	0	0
	Green Sunfish	0	10	10	10	10
		5	10	10	9	9
		10	10	10	9	9
		20	10	10	2	2
		30	10	10	0	0
	Bluegill (small)	0	5	5	5	5
		5	5	5	3	3
		10	6	6	6	6
		20	6	0	0	0
UDMH (200 ppm)	Green Sunfish	0	10	10	10	10
		10	10	10	10	10
		20	10	10	10	10
		30	10	10	10	9
		130	10	10	9	9
Pyridine Borane (100 ppm)	Green Sunfish	0	10	10	10	10
		10	10	10	9	8
		30	10	10	10	7
		205	10	10	8	7

* The fish were dipped for the indicated periods and then returned to the standard reference water.

/ Concentrations are given on a v/v basis.

b. Daphnia pulex

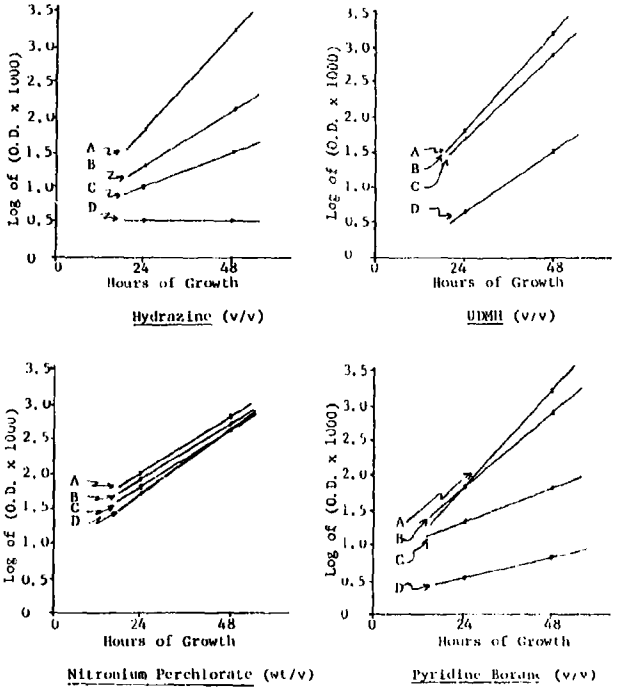
Adult Daphnia from an actively growing culture were added to 50 ml of a specific test solution in a 100 ml beaker. Mortality of the Daphnia was recorded at one hour by observation of dead organisms and at 24 hours by counting the living organisms during removal by pipette (Tables 22, 23, 24, 25 and 26).

c. Chlorella pyrenoidosa

Concentrations of the test chemicals were made using the modified Knops culture solution (Appendix A) as the make-up water.

Forty ml of the test solutions were placed in one inch diameter test tubes and the tubes inoculated with aliquots from an actively growing culture of a high temperature strain of Chlorella pyrenoidosa. The tubes for the hydrazine, UDMH and pyridine borane were aerated with five percent CO_2 in air. The nitronium perchlorate tubes were aerated with air. The cultures were grown under fluorescent lights at 74°F which is somewhat below the optimum growth temperature for the high temperature Chlorella. Growth of the

cultures was measured by reading optical density in a Bausch and Lomb Spectronic 20 colorimeter. The Chlorella growth data are presented in Figure 9.



Concentrations Added: A - control B - 1 ppm, C - 10 ppm, D - 100 ppm

FIGURE 9. Growth of Chlorella pyrenoidosa cultures in a modified Kropp solution at four nominal concentrations of hydrazine, UDMH, pyridine borane and nitronium perchlorate. Results are given as the log of the (OD x 1000).

2. Discussion

a. Animal Studies

All four of the chemicals tested are potentially serious pollutants for aquatic environments if discharged directly into the water, or if carried by direct runoff into a lake or stream. Where runoff into a lake or stream is delayed, or where the solution would percolate through soils, breakdown and adsorption should remove the pollution hazard. This should be true for both UDMH and hydrazine if they decompose as rapidly under natural conditions as they did under the conditions in these experiments.

1) Hydrazine

The TLm values shown in Table 26 compare closely

with those obtained in earlier experiments (ref. 12) where an alkaline water source was used.

The green sunfish and large bluegill had the highest tolerance of the fish species tested while channel catfish was the most sensitive species. The younger bluegill were more sensitive than the larger ones, as might be expected. Hydrazine, under the test conditions used, is by far the most toxic of the four chemicals tested. All TLm values for hydrazine were below 6 ppm.

The hydrazine breakdown in standard reference water, as shown in B-6, was paralleled by the results given in Table 27. At a concentration of 7.5 ppm, which is well above the TLm for goldfish in hydrazine, mortality was nearly complete at 24 hours in the fresh solution. When the solutions stood for 24 hours or longer before the fish were added, there was no mortality in a subsequent 96 hour exposure period.

The timed exposure experiments may be used to indicate that a relatively low hydrazine concentration moving in a water mass could cause extensive kill in a relatively short exposure time (Table 28).

2) Unsymmetrical Dimethyl Hydrazine

TLm values are comparable to those obtained in previous experiments (ref. 12) for Daphnia but are somewhat above those previously recorded for the goldfish. Goldfish and green sunfish had the highest tolerance levels while the channel catfish was the most sensitive species. Age and size affect sensitivity but not to the extent shown with hydrazine. The TLm values for UDMH were generally much higher than those for hydrazine.

The timed exposure experiments demonstrated that even at a concentration of 200 ppm, nearly four times the 48-hour TLm, there was no significant mortality in green sunfish exposed for over 2 hours (Table 28).

Experiments using the aged test solutions (Table 27) did not follow the breakdown curves for UDMH (B-6). Essentially the same mortality pattern was noted when the test solution was used at once or aged for 24 or 48 hours.

3) Pyridine Borane

Pyridine borane appears to be intermediate in toxicity between hydrazine and UDMH. Goldfish have the highest

tolerance and channel catfish are again the most sensitive.

The timed exposure would suggest that pyridine borane is not quickly ingested into green sunfish. About 50 percent mortality was noted in green sunfish when exposed for 2 1/2 hours at 100 ppm, which is over five times the 48 hour TLm.

Experiments using aged test solutions support the thesis that pyridine borane is not broken down in water solution. The toxicity of the 72-hour old pyridine borane solution was not significantly different from that of the fresh solution.

4) Nitronium Perchlorate

Results, Table 25, indicate rather strongly that neither the nitrate nor the perchlorate ions per se are toxic to the fish species at any of the concentrations used. Daphnia showed a high TLm (82 ppm). Toxicity noted is probably due to the hydrogen ion concentration.

b. Chlorella Studies

At the lowest concentration tested, 1 ppm, UDMH caused no decrease in growth of the Chlorella culture as compared to the control. Pyridine borane and nitronium perchlorate showed a slight decrease and hydrazine a marked decrease.

At the highest concentration tested (100 ppm) nitronium perchlorate gave only a slight decrease in growth, pyridine borane and UDMH showed a marked decrease, and hydrazine prevented growth completely.

In general, hydrazine was most detrimental to Chlorella growth and nitronium perchlorate least detrimental.

The results indicate that the use of Chlorella cultures may be an effective way to quantitatively evaluate the possible toxic effect of test chemicals.

F. General Discussion and Summary

The results of the three phases of research into the potential environmental pollution by hydrazine, unsymmetrical dimethyl hydrazine (UDMH), pyridine borane and nitronium perchlorate suggest that environmental conditions could be controlled or selected so that the test compounds would not be such serious contaminants.

Hydrazine and UDMH should be removed from any contaminated area within a rather short period of time if the rate of natural breakdown corresponds to the rate found under our experimental conditions. In the case of a spill, soils, even if low in clay, should hold both hydrazine and UDMH for short periods of time, sufficient for natural breakdown to occur. This would be true, in the case of hydrazine, only if the pure compound was diluted with water. If a large spill were to contaminate an aquatic area, there would be an initial high rate of kill. However, in stream contamination, this water mass should deteriorate with time. If the contaminated water could be contained for a period of time behind a holding dam, breakdown should naturally occur and the waters could then be slowly released. However, we have no experimental data on the breakdown of either hydrazine or UDMH under natural conditions.

The nitronium perchlorate should not be a pollutant except in the near vicinity of a heavy spill.

Pyridine borane should not act as a pollutant unless there was a large spill in an aquatic area. Holding stream waters and releasing them gradually so that a heavily contaminated water mass did not pass down the stream should be a workable control measure.

G. Recommendations for Future Study

The products of breakdown of UDMH and hydrazine need to be identified. Rates of breakdown in the current experiments do not correspond to breakdown rates reported at the Biomedical Laboratories at Wright-Patterson Air Force Base. Preliminary work indicates that copper may be a prime activating agent in the breakdown of both UDMH and hydrazine and that the copper is active at very low concentrations. This copper activation needs to be explored as well as other possible metal activators and oxidizing agents. The cause of UDMH and hydrazine breakdown needs to be detailed before the results of laboratory experiments can be used to predict toxicity reactions under natural conditions.

Natural water sources should be chemically analyzed and treated with UDMH and hydrazine to determine the effect of various water sources on the breakdown of UDMH and hydrazine. Some aquatic and plant studies should be done with several natural water sources.

Under conditions where breakdown does not occur, if these can be maintained, research needs to be continued in an effort to define long term effects of continual pollution by hydrazine, UDMH and especially some of the more toxic borane compounds. The long term effects of these chemicals on plant and aquatic life are as yet unknown.

The breakdown components of hydrazine and UDMH need further study. This is especially true for UDMH where the products formed have not been determined.

There is a very real need to study various mixtures of chemicals which would be present in missile fuels or exhaust gases. The interactions of gaseous and liquid toxicants have received far too little research attention. The air pollution aspects of this type of study could be very productive. Research to date has shown that the photochemical oxidation of hydrocarbon mixtures often produce substances more toxic than the original organic or inorganic toxicant. Nitrogen dioxide and olefinic hydrocarbons have been well studied in this respect.

H. References

1. Audrieth, L. F., and Betty Ogg, The Chemistry of Hydrazine, John Wiley and Sons, Inc., New York, N. Y., 1951.

2. Baltz, D. F. (Editor), Colorimetric Determination of Nonmetals, Interscience Publishers, New York, N. Y., pp 76-77, 1958.

3. Brindley, G. W., and M. Ruston, "Adsorption and Retention of Organic Materials by Montmorillonite," American Minerology, Vol 43, pp 627-640, 1958.

4. Callery Chemical Company, Amine Boranes, Tech. Bull. C-200, Callery, Pa., April 1958.

5. Callery Chemical Company, Nitronium Perchlorate, Tech. Bull. C-1200, Callery, Pa., April 1960.

6. Daniels, F., and R. Alberty, Physical Chemistry, John Wiley and Sons, Inc., New York, N. Y., 1956.

7. DeWerth, A. F., and R. E. Odom, A Standard Light-Weight

Growing Medium for Horticultural Specialty Crops, Texas Agric. Exp. Sta. Bull., MP-420, College Station, Texas, 1960.

8. Doudoroff, P. (Editor), "Bio-assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish," Sewage and Industrial Wastes, Vol 23, pp 1380-1397, 1951.

9. Gieseking, J. E., "The Mechanism of Cation Exchange in Montmorillonite-beidellite-nontronite Type of Clay Minerals," Soil Science, Vol 39, pp 1-11, 1939.

10. Grim, R. E., Clay Mineralogy, McGraw-Hill Book Company, Inc., New York, N. Y., 1953.

11. Hannan, D. G., "Boron Hydrides," National Safety News, pp 39-50, August 1961.

12. Heck, W. W., L. S. Bird, M. E. Bloodworth, W. J. Clark, D. R. Darling, and Martha B. Porter, Environmental Pollution by Missile Propellants, MRL-TDR-62-38, 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson AFB, Ohio, April 1962.

13. Hill, W. H., J. M. Merrill, R. H. Larsen, D. L. Hill, and J. F. Heacock, "Colorimetric Determination of Boron Hydrides by Means of Phosphomolybdic Acid," Industrial Hygiene Journal, pp 5-12, 1959.

14. Hoagland, D. R., and D. I. Arnon, "The Water Culture Method for Growing Plants Without Soil," California Agricultural Experiment Station, Circular No. 347, pp 31-32, Riverside, California, 1950.

15. Lange, W. A., Handbook of Chemistry, Handbook Publishers, Inc., Sandusky, Ohio, pp 108-110, 1956.

16. Manual on Industrial Water and Industrial Waste Water, Second Edition, American Society for Testing Materials, Committee D-19 on Industrial Water, Philadelphia, Pa., 1960.

17. Pinkerton, Mildred K., J. M. Lauer, P. Diamond, and A. A. Tamas, A Colorimetric Determination for 1,1-Dimethylhydrazine in Air, Blood, and Water, ASD-TR 61-708, Aerospace Medical Laboratory, Aeronautical Systems Division, Wright-Patterson AFB, Ohio, 1961.

18. Siegmund, J. M., R. B. Jackson, R. E. Ogden, and B. T. Poon, Research on Materials and Methods for Decontamination of Toxic Missile Propellant Spillage, ASD-TDR-62-64, Directorate of Materials and Processes, Wright-Patterson AFB, Ohio, 1962.

19. U. S. Dept. of Agric., "Diagnosis and Improvement of Saline and Alkaline Soils," Agric. Handbook No. 60, Washington, D. C., pp 83-147, 1954.

20. U. S. Dept. of Agric., "Manual for Testing Agricultural and Vegetable Seeds," Agric. Handbook No. 30, Washington, D. C., pp 88-106, 1952.

21. Watt, G. W., and J. D. Chrisp, "A Spectrophotometric Method for the Determination of Hydrazine," Anal. Chem., Vol 24, pp 2006-2008, 1952.

APPENDIX A

Preparation of Special Media

1. Peat-Perlite Potting Mix (ref. 7)

a. Ingredients

1) 35 liters of moist horticultural peat
2) 35 liters of moist horticultural perlite
3) 11 gm of KNO_3
4) 11 gm of $Ca(NO_3)_2$
5) 11 gm of KCl
6) 112 gm of 0-20-0 superphosphate
7) 337 gm of dolomite - $Mg(CO_3)_2$
8) 112 gm of gypsum - $CaSO_4 \cdot 2H_2O$
9) 14 gm of chelated iron (10.5 percent Fe)

b. Preparation

The peat and perlite is thoroughly moistened using a spray nozzle. Spread out 1/4 of the moist peat-perlite mixture; add 1/4 of the nutrient mixture and wet down with a fog nozzle; continue until all the ingredients are added; then, mix the layers together and keep moist when not in use.

2. Hoagland's Solution (ref. 14)

a. Stock Solutions

1) Molar solutions of the following major nutrients: KNO_3; $Ca(NO_3)_2$; $MgSO_4 \cdot 7H_2O$; and, $NH_4H_2PO_4$.

2) Supplementary solution: contains the following chemicals in one liter of water: H_3BO_3 (2.86 gm); $MnCl_2 \cdot 4H_2O$ (1.81 gm); $ZnSO_4 \cdot 7H_2O$ (0.22 gm); $CuSO_4 \cdot 5H_2O$ (0.08 gm); and, $H_2MoO_4 \cdot H_2O$ (0.02 gm).

3) Iron solution: contains 44 gm of an iron chelate (10.5 percent Fe) per liter of water.

b. Preparation

Add the following number of ml from each stock solution to one liter of water:

KNO_3 - 6 ml
$Ca(NO_3)_2$ - 4 ml
$MgSO_4 \cdot 7H_2O$ - 2 ml
$NH_4H_2PO_4$ - 1 ml
Supplementary - 1 ml
Iron chelate - 1 ml

3. Standard Reference Water (ref. 16)

a. Stock Solutions

1) Stock Solution 1. Dissolve 71.0 gm $MgSO_4 \cdot 7H_2O$, 6.5 gm K_2SO_4, and 0.2 gm $MnSO_4 \cdot 4H_2O$ in water and dilute to 1 liter.

2) Stock Solution 2. Dissolve 18.6 gm $CaCl_2 \cdot 2H_2O$ in water and dilute to 1 liter.

3) Stock Solution 3. Dissolve 25.0 gm $NaHCO_3$, 3.0 gm NH_4NO_3, and 1.1 gm $K_2HPO_4 \cdot 3H_2O$ in water and dilute to 1 liter.

4) Stock Solution 4. Dissolve 32.2 gm CaO in water and dilute to 1 liter. Bubble CO_2 gas through this mixture to make a $CaCO_3$ slurry.

5) Stock Solution 5. Dissolve 62.6 gm $Na_2SiO_3 \cdot 9H_2O$ in water and dilute to 1 liter.

6) Stock Solution 6. Dissolve 1.2 gm $FeCl_3 \cdot 6H_2O$ in water and dilute to 1 liter.

b. Method of Preparation

For each liter of standard reference water to be prepared, add 1 ml each of solutions 1, 2 and 3. Disperse pure CO_2 gas into this solution by means of a gas diffuser for 15 min. The pH of the solution at this point should be about 4.3. Add 1 ml of solution 4 to each liter of standard reference water, and introduce CO_2 gas until the solution becomes clear. The pH at this point should be about 5.1. Then diffuse compressed air through the solution for 25 min, to raise the pH to about 7.9. Add 1 ml each of solutions 5 and 6 to each liter of water, and aerate for 60 min. The final pH of 7.9 remains constant within 0.1 pH units.

4. Modified Knops Solution[1/]

The solution contained the following amounts of the listed chemicals in one liter of glass distilled water:

2.5 gm of $MgSO_4 \cdot 7H_2O$
1.25 gm of KNO_3
1.25 gm of KH_2PO_4
83.5 mgm of $CaCl_2$
114.2 mgm of H_3BO_3
49.8 mgm of $FeSO_4 \cdot 7H_2O$
88.2 mgm of $ZnSO_4 \cdot 7H_2O$
14.4 mgm of $MnCl_2 \cdot 4H_2O$
7.1 mgm of MoO_3
15.7 mgm of $CuSO_4 \cdot 5H_2O$
4.9 mgm of $Co(NO_3)_2 \cdot 6H_2O$
0.50 gm of EDTA - a chelating agent

This solution was adjusted to pH 6.8 with KOH.

[1/] Personal communication from Dr. Jack Meyer, Department of Biology, University of Texas, Austin, Texas.

APPENDIX B

Colorimetric Procedure for the Determination of 0.1-4 ppm of Hydrazine in Water[1/]

1. Reagents

a. Color developing reagent: Place one gm of p-dimethylaminobenzaldehyde into a 50 ml volumetric flask and make to volume with absolute ethyl alcohol. Agitate until dissolved. This reagent should be stable for 24 hours; however, if a distinct color change is noted (from very light brown to green), a new supply of reagent should be made.

b. Distilled water

c. Concentrated HCl

2. Procedure

Place the following in a 50 ml Erlenmeyer flask:

a. One ml of water sample containing hydrazine.

b. Three ml of color developing reagent, mix and allow to stand one minute.

c. Three ml of distilled water and mix.

d. One ml of concentrated HCl and mix. Allow to stand five minutes for color development.

e. Prepare blank using four ml of distilled water, three ml of color developing reagent and 1 ml of concentrated HCl.

f. Read samples at 460 mμ in a colorimeter or spectrophotometer.

1/ Lauer, J. M., "Quantitative Colorimetric Procedure for the Detection of Microgram Quantities of Hydrazine in Water and Serum," Obtained by personal communication with Mr. Philip Diamond, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio, 1961.

3. Comments

a. The times specified above allow the constituents to react completely. The times are not critical and it should not be assumed that the colored ion is in any way unstable. No change in color was observed over a 24-hour period in samples analyzed using the above procedures.

b. Any substance with a NH_2 group will condense with p-dimethylaminobenzaldehyde and in acid medium will form a colored quinoloid type ion.

c. The sample must be read immediately after placing in the sample holder as the temperature of the sample and reference increase in temperature with time resulting in error. The same reasoning applies for removing the reference sample between readings.

APPENDIX C

X-ray Diffraction and Differential Thermal Analyses[1/]

1. Introduction

A brief explanation of the use of X-ray diffraction on soil analysis will help to clarify the results presented in this report. The X-ray diffraction patterns are produced by the following procedure:

a. Forty kilovolt X-rays, with a wave length of 1.5405 Å are produced from a copper target.

b. The 1.5405 Å X-rays strike a crystal orientated clay sample.

c. The orientated clay sample refracts the X-ray beam according to Braggs' formula ($n\lambda = 2\ d \sin\theta$).

d. A geiger counter then scans the refracted X-ray beam and a recorder reproduces the intensity of the refracted X-ray beam at the desired angles.

e. By using Bragg's formula, the angles of high intensity are then converted to Angstrom-unit spacings.

f. The spacings of the refracting atomic planes are then used to identify the clay minerals. The spacings of the atomic

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1/ Detailed information on X-ray diffraction can be found in the following publications:

Brindley, G. W., X-ray Identification and Crystal Structures of Clay Minerals, The Mineralogical Society, London, England, 1951.

Brown, G. (Editor). The X-ray Identification and Crystal Structures of Clay Minerals, The Mineralogical Society, London, England, 1951.

Mackenzie, R. C., The Differential Thermal Investigation of Clays, The Mineralogical Society of London, London, England, 1957.

planes also can be used, in the case of certain clay minerals, to determine the space occupied between the clay layers by adsorbed materials.

Six diffractograms are presented for each soil. The $MgCl_2$-ethylene glycol and KCl saturated diffractograms were used to identify the clay minerals. The other four diffractograms were used to determine the adsorption of the test compounds. If the compounds tested were adsorbed on the clay complex, they would form individual layers of approximately 3.4 Å in thickness for hydrazine, 4.9 Å in thickness for UDMH, 3.4 to 5.7 Å in thickness for pyridine borane, and 3.0 Å thickness for water.

It also should be pointed out that the X-ray analyses are of much more value when they are supported by other research methods such as differential thermal analysis and actual chemical exchange analysis. Both were used to support the X-ray diffraction studies and from which conclusions for the study were formulated.

It has been stated previously in this paper that important differences exist in the montmorillonite and kaolinite clay crystals. It should be pointed out and emphasized that montmorillonite and certain clay-size micas are the only clay minerals that will expand to permit cations and organic materials to penetrate within the crystal lattice. In the X-ray diffractograms of the clays tested, it can be observed that none of the basal spacings, except those of montmorillonite (clay mineral), shifted as a result of the treatments which were applied.

2. Discussion of the X-ray Diffractograms of the Soils Under Study

a. Identification of Clay Minerals

1) Kaolinite: Figure 10 is a good example of a diffractogram for a well ordered, crystalline, relatively-pure kaolinite clay. The 7.12 Å peak for the (001) first order spacing agrees with the 7.13 Å structure of kaolinite given by Grim (ref. 10). The (002) second order peak at 3.55 Å is merely one-half of the first order peak of 7.12 Å. A third order peak also was observed at one-third of 7.12 Å. In some cases a highly ordered crystal can produce as many as 12 orders which can be read on a diffractogram; however, the first two to three orders are generally diagnostic in determining the identity of a clay mineral.

It is important to note that the kaolinite peaks were not shifted by any of the adsorbed chemical compounds; therefore, any adsorption had to occur at the edges of the plates

(kaolinite crystals).

2) Montmorillonite: Figure 11 shows X-ray diffractograms of well-ordered crystalline montmorillonite which contains a small amount of quartz and feldspar. The KCl saturated sample has a peak at 12.8 Å. Since the completely dehydrated montmorillonite crystal is approximately 9.6 Å, this indicates that there is one layer of water with the potassium ion in the interlayer spacing. The fact that the spacing shifted to 17.3 Å with the $MgCl_2$-ethylene glycol treatment is a diagnostic indication of montmorillonite. The double charge of the magnesium ion, coupled with the adsorption of the relatively large molecule of ethylene glycol, expands the montmorillonite crystal to the characteristic 17.0 Å spacing.

The 3.32 Å spacing is low for a (3.35 Å) quartz peak. This peak is probably due to a combination of third order (10.0) illite peak and a quartz peak. Even though the peak is well defined, there is probably little quartz or illite present. The height of the peaks depends upon the order of the crystalline material, as well as the amount of the material present; therefore, the high degree of order in the quartz crystal produces a relatively strong peak, even though the amount of quartz present may be low (2-3%). The same reasoning is true with regard to the 3.19 Å feldspar peak. The quantity of feldspars present is though to be very small, even though there is a well defined peak. An important factor that has a tendency to increase the height of the peaks in the quartz and the feldspar range is the fact that lower order reflections from other clay minerals also fall in this range.

3) Yolo: Figure 12 indicates that the clay fraction of the soil contains montmorillonite (16.7 Å-Mg and 13.7 Å-K), illite (10.3 Å first order and 5.00 Å second order), kaolinite (7.30 Å first order and 3.57 Å second order, and quartz (3.35 Å).

4) Houston: Figure 13 indicates that montmorillonite (18.0 Å-Mg and 13.1 Å-K) is the predominant clay mineral in the Houston Black clay soil. There are small amounts of kaolinite (7.23 Å first order and 3.56 Å second order) and quartz (3.35 Å) present.

5) Lufkin: Figure 14 indicates that montmorillonite (17.5 Å-Mg and 14.0 Å-K) is the predominant clay mineral in the sample of Lufkin soil studied; however, there is also an indication of a mixture of other materials present. Some kaolinite (7.23 Å first order and 3.57 Å second order) and quartz (3.36 Å) are also present.

6) Aiken: According to Figure 15, the clay fraction of the Aiken soil consists largely of a mixture of poorly ordered materials. Some of the probable minerals present are vermiculite (14.2 Å-K and 15.5 Å Mg), chlorite (14.2 Å), kaolinite (7.22 Å to 7.30 Å), magnetite (4.84 Å, in sufficient quantities to be easily detected on the laboratory magnetic stirrer), ∝-cristobalite (4.05 Å and 3.14 Å), and quartz (3.35 Å).

b. X-ray Diffraction Analysis of the Test Materials

1) Hydrazine and UDMH

Other than montmorillonite, a change in the clay minerals due to the treatment with hydrazine and UDMH, was not detected.

The spacing for hydrazine saturated clays with a montmorillonite component ranged from 12.8 Å to 13.3 Å. This range indicates that the lattice of montmorillonite (9.6 Å - 10.0 Å) has adsorbed one molecular layer of hydrazine (3.4 Å).

The spacing for UDMH saturated montmorillonite ranged from 13.5 Å to 15.1 Å. Even though the montmorillonite lattice (9.6 Å-10.0 Å) and one layer of UDMH (4.9 Å) could logically fall in this range, an explanation for the wide range is necessary. From observation of the time required to adsorb UDMH on the test clays, it has been concluded that the differences in the spacings are due to different amounts of adsorption. In the case of the 13.5 Å spacing, the adsorption of one layer of UDMH molecules was not complete. The molecular structure could accomodate the UDMH molecule in a close packing arrangement. The 15.1 Å spacing indicates that one layer of adsorbed UDMH molecules was complete.

2) Pyridine Borane and Nitronium Perchlorate

Without other research data, it would be easy to arrive at erroneous conclusions about the adsorption of pyridine borane and nitronium perchlorate from the X-ray diffractograms. Chemical exchange data, presented in another section of this report, rules out any adsorption of the two test chemicals involved; therefore, an explanation for the montmorillonite spacings can easily be concluded.

Nitronium perchlorate was used at a concentration of 10,000 ppm. Upon adding water, nitronium perchlorate would form a 0.136N (Normal) acid at this concentration. The hydrogen ion with one layer of water would exchange onto the clay and produce the spacings that are observed in the X-ray diffractograms.

Pyridine borane had no effect on the spacing. The spacings of the pyridine borane treated soil were due to the original, natural-occurring cations in the soil.

c. Differential Thermal Analyses (DTA) of Test Materials

1) Explanation of the Differential Thermal Method of Analysis

The differential thermal method of analysis detects physical and chemical changes of a substance by recording the exothermal and endothermal changes in enthalpy as the temperature is changed. A composite of montmorillonite and kaolinite DTA patterns is shown in Figure 16. In the case of kaolinite and montmorillonite, the magnesium saturated curves represent the normal DTA patterns.

2) Montmorillonite (Figure 16)

In the case of the magnesium saturated montmorillonite clay, the first endotherm (180°C) represents the loss of free-water adsorbed on the clay. The second endotherm (695°C) represents the loss of OH water (OH's in the clay crystal). The third endotherm (865°C) and the exotherm at 900°C represents the crystal rearrangement into another high-temperature phase mineral.

The DTA curve of the hydrazine saturated montmorillonite clay indicates an endothermic peak at 140°C. This endothermic peak is probably due to the evaporation of hydrazine and some readsorbed water. The exothermic peak (220°C) indicated that hydrazine is oxidized and removed from the clay lattice at this temperature. A sample of thermally inert Al_2O_3 was treated with pure hydrazine and the resulting DTA pattern (not shown in Figure 16) indicated an endothermic peak at the boiling point of hydrazine (113.5°C). No other peaks were indicated, so it was assumed that the hydrazine molecules adsorbed at the edges of the Al_2O_3 particles were held so loosely that they were lost by evaporation before the exothermic oxidation, at 220°C, could take place. However, in the clay lattice the hydrazine molecules were probably held in place until the temperature reached the decomposition or oxidation temperature.

The DTA curve of the UDMH saturated montmorillonite clay indicates an endothermic peak at 130°C. This endothermic peak is probably due to the evaporation of UDMH and some readsorbed water. The exothermic peak (610°C) is broad and extends to such a high temperature that it is concluded that the UDMH

molecule must be retained by the clay with a much greater tenacity than the hydrazine molecule. The broadness of the peak also indicates that a considerable amount of UDMH was adsorbed on the clay.

A sample of thermally inert Al_2O_3 was treated with pure UDMH and the resulting pattern (not shown in Figure 16) indicated a diffuse endothermic peak which extended through the boiling point of UDMH (62.5°C) and the boiling point of water (100°C). No exotherms were observed with the UDMH saturated Al_2O_3, so it can be concluded that some UDMH or derivatives thereof were probably held in the clay lattice up to 610°C without being decomposed or oxidized. The UDMH interaction with montmorillonite is of considerable interest since most compounds show little tendency of adsorption with montmorillonite, other than through the exchange phenomenon.

3) Kaolinite (Figure 16)

In the case of the magnesium saturated kaolinite clay, the endothermic peak (595°C) is due to the loss of OH water (OH's in the clay crystal). The exotherm at 980°C. results when the kaolinite mineral is transformed into another high-temperature phase mineral.

There were no exotherms or endotherms associated with UDMH or hydrazine and kaolinite clay. This would indicate no adsorption of these compounds on kaolinite. These latest findings give reason for some doubt concerning the chemical exchange data of kaolinite in this paper. With the DTA curves in mind, it would be safe to say at this time that most of the reported adsorption of UDMH (129.2 meq/100 g maximum adsorption) and hydrazine (30.3 meq/100 g maximum adsorption) was likely to be due to decomposition or physically occluded material within the soil aggregates.

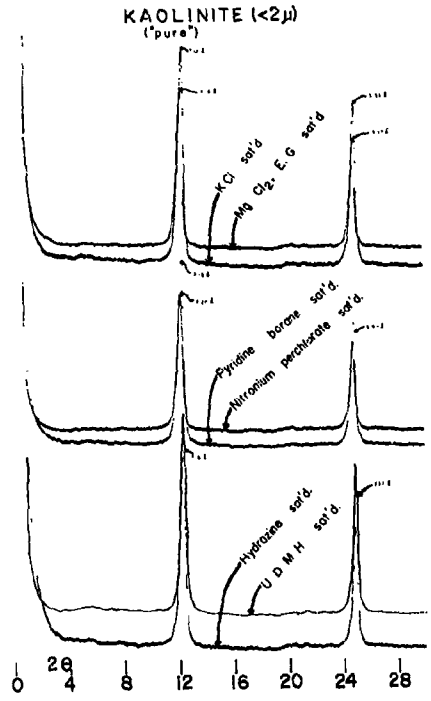

Figure 10. X-ray diffraction patterns for "pure" Kaolinite.

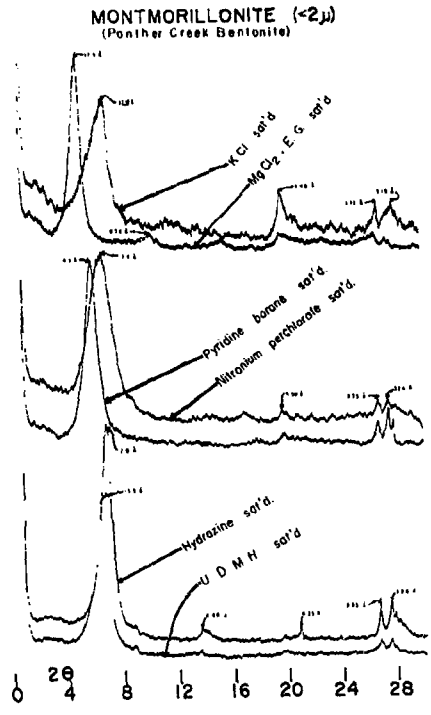

Figure 11. X-ray diffraction patterns for "pure" Montmorillonite.

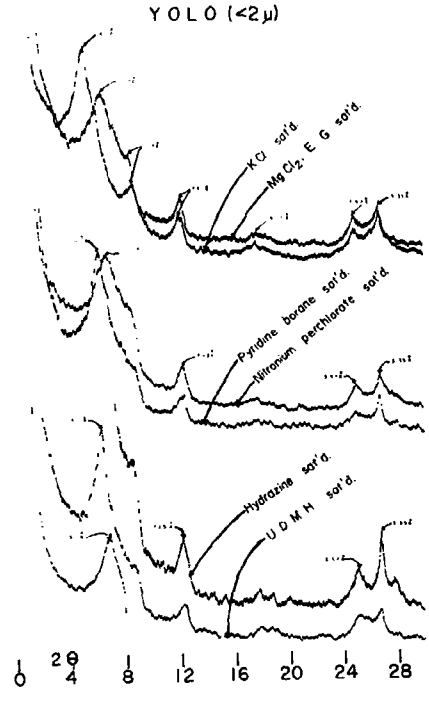

Figure 12. X-ray diffraction patterns for clay of the Yolo soil.

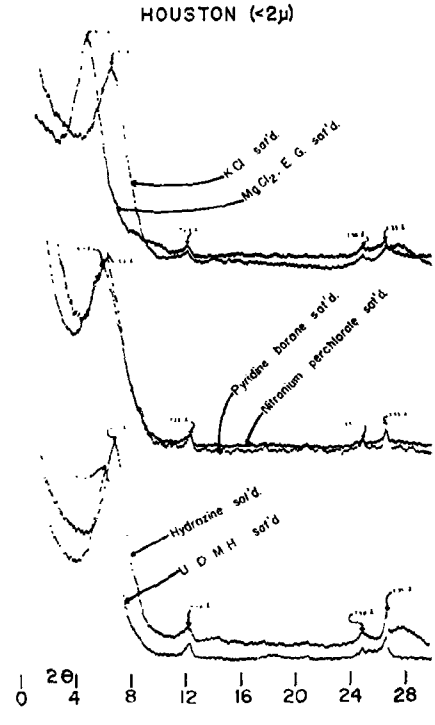

Figure 13. X-ray diffraction patterns for clay of the Houston soil.

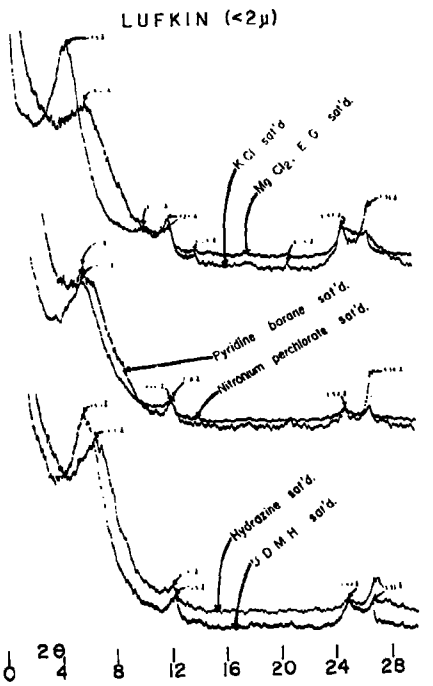

Figure 14. X-ray diffraction patterns for clay of the Lufkin soil.

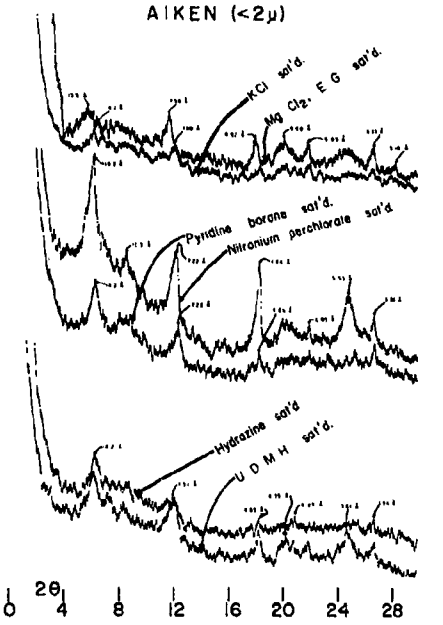

Figure 15. X-ray diffraction patterns for clay of the Aiken soil.

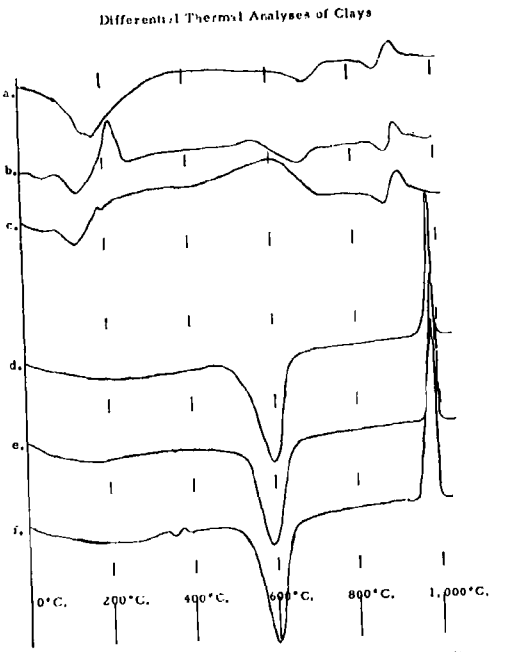

Figure 16. The differential thermal analyses of saturated montmorillonite and kaolinite clays.

a. Mg saturated montmorillonite, b. Hydrazine saturated montmorillonite c. UDMH saturated montmorillonite, d. Mg saturated kaolinite, e. Hydrazine saturated kaolinite, f. UDMH saturated kaolinite.

APPENDIX D

Statistical Data from Germination Studies

TABLE 29

Mean Squares for the Effect of Hydrazine, UDMH, Pyridine Borane and Nitronium Perchlorate on the Germination of Squash, Peanut and Corn***

Source of Variation	Degrees Freedom	Mean Squares			
		Hydrazine	UDMH	Pyridine Borane	Nitronium Perchlorate
Replications	3	0.76	1.31*	.06	0.42
Treatments	14	8.78**	6.03**	2.22**	0.47
Species	2	0.62	8.82**	10.95**	1.95*
Concentrations	4	20.77**	5.61**	.94	0.65
Species x Conc.	8	5.05**	6.04**	.68	0.30
Error	42	1.25	0.42	.56	0.41
Total	59	3.03	1.80	.93	0.42

* Significant at the .05 level.

** Significant at the .01 level.

*** This presents results of the 3 x 5 factorial design of the 48 hour observations of the four test chemicals after neutralization, at five concentrations.

Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio. Rpt. No. AMRL-TDR-63-75. ENVIRONMENTAL POLLUTION BY MISSILE PROPELLANTS. Final report, Aug 63, ix + 79 pp. incl. illus., tables, 24 refs. Unclassified report

Experimental procedures were developed to study the effects of hydrazine, unsymmetrical dimethylhydrazine (UDMH), pyridine borane, and nitronium perchlorate on plant growth and development, soil and soil structure, and aquatic organisms. Plant growth and development research included: seed germination, seed growth, and treatment of plants in water culture, by sprays,

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2. Toxicity
3. Environment
4. Missile Propellants
I. AFSC Project 6302, Task 630204
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